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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Nancy T. Chang

Serial No.: 659,339

Filed: October 10, 1984

Title: CLONING AND EXPRESSION OF HTLV-III DNA

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 5-14-86

Hamilton, Brook, Smith & Reynolds

Ellen Kitrosser
Signature

5-14-86
Date

DECLARATION OF NANCY T. CHANG

The Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Nancy T. Chang, of 7405 Brompton St., Houston, Texas 77025, declare:

1. I am an inventor of the subject matter described and claimed in the above-identified application. When the invention was made, I was

Affidavit Exhibit 2
CHANG ET AL.
Interference No. 103,659

Associate Research Director in Molecular Biology at Centocor, Incorporated, Malvern, Pennsylvania (Centocor), assignee of the subject application. Currently, I am an Associate Professor of Medicine at Baylor College, Houston, Texas.

2. At the time the application was filed, Dr. Robert C. Gallo and Dr. Flossie Wong-Staal were not designated co-inventors when, in fact, they were co-inventors and should be so designated.

3. The above-identified application discloses and claims methods for cloning and expressing subgenomic fragments of HTLV-III cDNA; HTLV-III cDNA fragments and immunoreactive HTLV-III polypeptides encoded thereby; and methods of detecting antibody against HTLV-III employing the polypeptides.

4. The experimental work described in the application began at Centocor upon receipt of genomic HTLV-III DNA from the laboratories of Dr. Gallo and Dr. Wong-Staal. Dr. Gallo and Dr. Wong-Staal supplied a recombinant phage (designated λBH 10) consisting of the genomic HTLV-III cDNA recombined with a phage vector. The HTLV-III cDNA insert was excised from λBH 10 and fragmented and the subgenomic fragments were cloned and expressed in host cell systems as described in the application. All of the experimental work described in the application was done at Centocor, either by me or by laboratory assistants working under my direction and supervision. However, at various times before the experimental work and during its progress, Dr. Gallo, Dr. Wong-Staal and I discussed the strategy

for the cloning and expressing of the viral cDNA. The experimental work proceeded along the lines we discussed; thus Dr. Gallo and Dr. Wong-Staal contributed signficantly to the cloning and expression of the HTLV-III cDNA.

5. On August 22, I prepared a document which described the experimental work accomplished up to that time. The document was sent to Centocor's patent law firm, Hamilton, Brook, Smith & Reynolds (HBS&R), as an "invention disclosure" (Exhibit A). Because all of the work described in the "invention disclosure" document was done at Centocor and because of my incomplete understanding of the law of inventorship, I did not designate Dr. Gallo or Dr. Wong-Staal as "inventor" on this document.

6. Subsequently, Centocor decided to have a patent application prepared and filed by HBS&R. Because of the imminent publication of an article disclosing work relating to the invention, there was great urgency to file the application. On October 8, 1984, I met with Centocor's patent attorneys to supplement information contained in the "invention disclosure" document (Exhibit A) for completing of a patent application. At this meeting, all of my time was devoted to explanation and discussion of the highly technical and complex subject matter necessary to prepare the application. The subject Application, Ser. No. 659,339 was filed on October 10, 1984.

7. On January 23, 1985, a continuation-in-part application was filed to cover additional experi-

mental work which had been done since the earlier application was filed. The inventorship error was repeated.

8. The possibility of an error in inventorship was first raised by Dr. Gallo in a letter to me dated July 25, 1985 (Exhibit B). Shortly thereafter, Centocor management initiated an investigation into the facts surrounding the invention and authorized HBS&R to do the same (Exhibit C). After a preliminary investigation, Centocor management made a tentative response to Dr. Gallo on September 16, 1985 (Exhibit D). However, pursuant to Centocor's stated desire to have the patent legally drawn a thorough investigation was made. During this investigation, I informed HBS&R of the full extent of Dr. Gallo's and Dr. Wong-Staal's collaboration with me regarding conceptual aspects of the claimed subject matter. After consideration, HBS&R concluded that Dr. Gallo and Dr. Wong-Staal should be designated as co-inventors because of their conceptual contributions.

9. My earlier failure to indicate the contributions of Robert C. Gallo and of Flossie Wong-Staal was unintentional.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nancy T. Chang

Nancy T. Chang

Feb. 23, 1986

Date

EXHIBIT A

THE CHARACTERIZATION AND PRODUCTION OF HTLV-III GENES AND PROTEINS BY GENETIC ENGINEERING METHODS

Nancy T. Chang

Centocor, Inc.

244 Great Valley Parkway

Malvern, PA 19355

August 22, 1984

Nancy T. Chang
Aug. 27, 1984

8/27/1

Witnessed by

Aug 27, 1984

Documentary Exhibit 5
CHANG ET AL.
Interference No. 103,659

Diagnostic and Vaccine Developments for AIDS

Human T Cell Leukemia Virus type III (HTLV-III), also named Lymphoadenopathy Virus (LAV), was isolated from the peripheral blood or lymphoid tissues of patients with Acquired Immune Deficiency Syndrome (AIDS). Recent studies of R. Gallo's group and of L. Montagnier's group indicated that the sera from over 80% of AIDS and pre-AIDS patients contain antibodies specific for the viral envelope and core proteins of HTLV-III. This and other evidence strongly suggests that HTLV-III is the cause of infectious AIDS, giving hopes that the diagnosis, preventive vaccine, and even therapy for AIDS can soon be developed.

Because AIDS can be transmitted through blood transfusions, an assay that detects HTLV-III infection is important not only for diagnosing patients but also for screening blood that might be contaminated with the virus. NIH and several commercial firms, including Centocor, are now developing immunoassay kits employing inactivated, disrupted HTLV-III as the solid-phase immunoadsorbent for the detection of antibodies against HTLV-III antigens in serum or blood.

Genetic Engineering Approach

Another approach for the detection of and the vaccination against HTLV-III infection is the employment of genetic engineering methods. In this approach, the proviral genes integrated into host cell DNA are molecularly cloned. The nucleotide sequence of the molecular cloned provirus is determined. The viral nucleotide sequence information will be directed to design and engineer HTLV-III-specific peptides and DNA probes using recombinant DNA technology or synthetic peptide chemical synthesis methods. These products are then explored for use in the diagnosis of HTLV-III infections by measuring specific antibody to the viral peptides or HTLV-III-specific RNA or DNA. The peptides, especially the gag and env related peptides may also be used as vaccines for the prevention of AIDS.

More specifically, the env and gag genes, which encode the envelope and core proteins of HTLV-III, respectively, are subcloned into various bacterial or mammalian expression vectors. These expression vectors contain all the necessary controlling elements for the production of the fused HTLV-III env gene in recombinant plasmids bearing host cells. Expression of the HTLV-III related peptide in the foreign host cells can be detected by binding of HTLV-III specific antibody in the AIDS patient serum or hyperimmune serum raised against purified virus. Although the env and gag products are of primary interest for diagnostic and vaccine purposes, the other two genes encoded by HTLV-III, pol and Px are important for understanding the biology of this retrovirus. These genes will be studied as well.

The genetic engineering approach offers a few advantages over the conventional one, which involves growing HTLV-III in cell cultures. For example, in the manufacturing process, because viral antigens are not infectious, working personnel are not exposed to the hazardous virus and the facility requirement will be less stringent than that for virus production. Also, the envelope and core proteins are the dominant immunoreactive viral antigens, immunoadsorbents with the purified viral proteins may offer more antibody-adsorbing capacity and higher sensitivity than those with whole virus. Immunoassays employing envelope and core proteins separately can detect antibodies against envelope and against core proteins. The antibody profile (concentrations and proportions) may reveal certain natures of the disease yet to be discovered. Furthermore, a protein vaccine using purified viral proteins (env or core gene product) will not have the risk of viral infectivity.

Centocor's First Footstep in HTLV-III Molecular Biology Work

As soon as we obtained the information in early May, 1984, that HTLV-III was isolated from AIDS patients and shown convincingly to be the cause of AIDS and that antibodies against HTLV-III antigens were found in over 85% of AIDS and Pre-AIDS patients. I decided to use the genetic engineering approach to develop diagnostic assays for AIDS. On May 10, 1984, Tse Wen Chang, Michael Wall and myself went to Biotech Corporation, Rockville, Maryland, to meet Dr. Robert Ting (Chairman of Biotech) to discuss the collaboration between Centocor and Biotech about coating polystyrene beads with inactivated disrupted HTLV-III. In that meeting, I expressed my interest to clone and

express HTLV-III genes and to use the expressed proteins for diagnostic and vaccine products. Dr. Ting was impressed with our expertise in Molecular Biology and introduced me to Dr. Flossie Wong-Staal, a key associate of Dr. Robert Gallo, with whom he had been collaborating on certain aspects of HTLV-III work. Our collaboration with the NCI group started on that day. We returned to Centocor with E. coli clones encoding segments of HTLV-II DNA. At that time, HTLV-III DNA had not been cloned.

The collaboration between Centocor and the NCI group went on very nicely. On July 5, we visited Dr. Wong-Stahl reporting our progress on HTLV-II and proposing our strategy on HTLV-III. We obtained λ clones harboring a segment of HTLV-III DNA on July 20, 1984. Our work on HTLV-III started on that day.

Centocor's Progress Update

We now have E. coli plasmid clones containing various portions or entire genome of HTLV-III. We have sequenced a segment (about 3500 base pairs long) of HTLV-III genome encoding most of the env gene. We have also cloned HTLV-III DNA in several expression host-vector systems and obtained several clones that can be induced to synthesize polypeptides encoded by the inserted HTLV-III DNA. Efforts are being made to test the reactivity of these polypeptides with antibodies from AIDS patients. When we identify clones that produce polypeptides demonstrating good reactivity with the antibodies, we will produce the polypeptide in large quantities and use it in immunoassay development. We also plan to clone and express the gag gene in a few weeks.

Plans are also being made to transfect mammalian cells with the E. coli cloned env and gag DNA's.

The Application of HTLV-III Related Peptides or Proteins

The viral envelope and core related peptides produced by the env and gag clones, either separately or combined, can be coated or conjugated noncovalently or covalently onto solid phase, such as PVC plate or polystyrene beads to be used as immunoadsorbent for antibodies against them. These solid phase immunoadsorbents are the key components in the immunochemical assays for HTLV-III-specific antibodies, using tracers such as goat anti-human immunoglobulin or protein A that are conjugated with radioactive isotopes such as ^{125}I, or enzymes such as peroxidase or alkaline phosphatase.

The proteins can also be used to prepare vaccine against HTLV-III, which should be useful for high-risk populations, such as homosexual males and recipients of frequent blood transfusions. The genetic engineered envelope and core proteins can also be used as an immunogen to prepare monoclonal or polyclonal antibodies. These antibodies can be employed in immunochemical assays for the detection of viral antigens in serum, blood, lymphocytes, or other tissues of AIDS or pre-AIDS patients.

The nucleotide sequences of HTLV-III env and gag genes yield information about the amino acid residue sequences of the envelope and core proteins.

Artificially synthesized segments of polypeptides according to the sequences may offer potential in diagnostic assays and in vaccines.

The cloned HTLV-III and its sequence can also be used to prepare DNA probes for the detection of HTLV-III RNA, proviral DNA, or encoded mRNA in the lymphocytes, or other tissues of patients.

EXHIBIT B

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20205
Building : 37
Room : 6A09
(301) 496-6007

July 25, 1985

Dr. Nancy Chang
Assistant Research Director
Molecular Biology
CENTOCOR
244 Great Valley Parkway
Malvern, PA 19355

Dear Nancy:

We are pleased that our collaborative efforts are making progress. Your synthesis of HTLV-III env gene products using our HTLV-III DNA clone is encouraging. We are beginning to use these in our larger NCI vaccine research development effort.

However, it has come to our attention that some time ago your organization filed a patent on the synthesis and uses of the expressed products from our HTLV-III DNA clones which were designated for collaborative research. We found out that our names are not included on the patent, despite the fact that your use of the clone was indispensable to your part of the effort.

We assume that this was an oversight. We would like to ask that your patent be modified and that our names be added to your patent application. We feel that a formal recognition of our contribution is integral and that the inclusion of our names is only fair.

Sincerely yours,

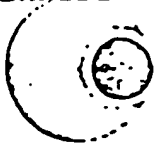
Robert C. Gallo, M.D.

RCG/PF/bj

cc Dr. Chabner
Dr. DeVita
Dr. Fischinger
→Dr. Harmison
Dr. Sliski
Dr. Wall

Documentary Exhibit 6
CHANG ET AL.
Interference No. 103,659

EXHIBIT C



CENTOCOR

244 GREAT VALLEY PARKWAY. MALVERN. PA 19355. (215) 296-4488
TELEX: 834823 CENTOCORMARN
FAX: 215-644-7558

August 5, 1985

David Brook, Esquire
Hamilton, Brook, Smith & Reynolds
Two Militia Drive
Lexington, Massachusetts 02173

Dear David:

I will respond to Dr. Gallo at the National Institutes of Health upon my return, August 20, 1985. In the meantime please discuss this matter with Nancy Chang regarding the facts surrounding this invention.

I believe Dr. Gallo mixes up inventorship with contribution. This issue is politically sensitive and I may wish to compromise. I will also discuss this with Dr. Lawless at Du Pont. Du Pont is licensed by the government.

Sincerely,

Hubert J.P. Schoemaker for

Hubert J.P. Schoemaker, Ph.D.
President

HJPS:so'h
attachment
cc: Dr. Nancy Chang
Dr. Gregory Lawless

end the harassment." *Ellison*, 924 F.2d at 881–82. Obviously, not all harassment warrants dismissal of the harasser. *Id.* (citing *Barrett v. Omaha Nat. Bank*, 726 F.2d 424, 427 (8th Cir.1984)). Rather, the remedy should be "assessed proportionately to the seriousness of the offense." *Id.* (citing *Dornhecker v. Malibu Grand Prix Corp.*, 828 F.2d 307, 309 (5th Cir.1987)). In essence, the reasonableness of a remedy depends on its effectiveness in ending the harassment. *Id.* at 882.

[22] Here, there is no dispute that Schlage knew of the alleged harassment of Pereira through Pereira's own repeated complaints. Schlage does, however, claim that after each of Pereira's complaints it investigated and counseled and/or warned the employees alleged to have harassed Pereira. According to Schlage, Pereira's accusations were simply never substantiated by any other evidence uncovered in their investigations.

In response, Pereira claims that her complaints were repeatedly ignored and/or dismissed. For example, according to Pereira, Bob Stanley said he could do nothing about her co-workers' language unless it was spoken in English. Pereira Dec. at 4. Furthermore, during one conversation, Kathy Jaramillo allegedly told Pereira that her accusations were "crazy". Pereira Dec. at 10. It is also undisputed that Schlage never attempted to institute formal disciplinary action against any of Pereira's alleged harassers or to transfer them to a different department where they would not come into contact with Pereira.

Therefore, issues of genuine fact remain as to whether Schlage's investigations, counseling, and warnings were sufficient remedial actions to excuse Schlage from liability.

B. *Retaliation*

[23] Although the language of Title VII and Cal. Gov't Code § 12940 differs slightly, their "antidiscriminatory objectives and the overriding public policy purposes are identical and [courts] refer to ... federal decisions where appropriate." *County of Alameda v. Fair Employment & Housing Commission*, 153 Cal.App.3d 499, 504, 200 Cal.Rptr. 381 (1984). The same issues of fact that remain on Pereira's federal claim for retaliation are applicable to her state law claim as well. Therefore summary judgment is not appropriate on this claim either.

CONCLUSION

Based on the foregoing, Schlage's motion for summary judgment is GRANTED on Pereira's Title VII claim for hostile working environment, and DENIED on her Title VII claim for retaliation and her state law claims for both hostile working environment and retaliation. Pereira's cross-motion for summary judgment is DENIED.

Schlage has also made a motion to continue the trial date. Accordingly, the trial date is vacated and new dates will be set by the Court.

IT IS SO ORDERED.

CHIRON CORPORATION, Plaintiff,

v.

ABBOTT LABORATORIES, Defendant.

No. C–93–4380 MHP.

United States District Court, N.D. California.

Sept. 14, 1995.

Patentee brought action against competitor, alleging infringement of its patent pertaining to immunoassay test for human immunodeficiency virus (HIV), and competitor raised inequitable conduct and prior invention defenses. On cross-motions for summary judgment on those defenses, the District Court, Patel, J., held that: (1) evidence raised genuine issue of material fact as to whether patentee submitted declaration to patent examiner that contained material misrepresentations regarding scope of prior art, precluding summary judgment on alleged in-

fringer's inequitable conduct defense; (2) immunoassay test for HIV was not conceived of by patentee for priority purposes when patentee had idea for recombinant immunoassay based on "env region" polypeptides; (3) evidence raised genuine issue of material fact as to whether patentee's competitor successfully reduced invention to practice prior to patentee, precluding summary judgment on alleged infringer's priority defense; and (4) patent application purporting to set forth HIV immunoassay did not meet statutory enablement requirement, and thus could not constitute constructive reduction to practice for purposes of priority defense.

Motion granted in part and denied in part.

1. Federal Civil Procedure ⇐2544

Where moving party has burden of proof on claim or defense raised in summary judgment motion, it must show that undisputed facts establish every element of claim or defense. Fed.Rules Civ.Proc.Rule 56, 28 U.S.C.A.

2. Patents ⇐323.2(4)

When defendant in patent infringement case moves for summary judgment on affirmative defense, elements of which defendant must prove by clear and convincing evidence, nonmoving party must simply produce enough evidence to allow for actual trier of fact to find that there is not clear and convincing evidence. Fed.Rules Civ.Proc.Rule 56, 28 U.S.C.A.

3. Federal Civil Procedure ⇐2543, 2552

Court's function on motion for summary judgment is not to make credibility determinations, and inferences to be drawn from facts must be viewed in light most favorable to party opposing motion. Fed.Rules Civ. Proc.Rule 56, 28 U.S.C.A.

4. Patents ⇐97, 312(4)

In order to prevail on inequitable conduct and prior invention defenses, alleged patent infringer must prove those defenses by clear and convincing evidence. 35 U.S.C.A. § 282.

5. Patents ⇐312(4)

Party asserting inequitable conduct defense in patent infringement case bears burden of proving both intent and materiality by clear and convincing evidence.

6. Patents ⇐97

Finding of materiality alone is insufficient to support finding of intent to deceive Patent and Trademark Office (PTO), which is separate element of inequitable conduct defense in patent infringement case; thus, in attempting to prove inequitable conduct, accuser may not rely solely on materiality of information allegedly withheld. Practice Rules in Patent Cases, § 1.56(a), as amended, 35 U.S.C.A.App.

7. Patents ⇐97

Gross negligence is not in and of itself sufficient to establish inequitable conduct in patent infringement case; rather, deceitful intent must be shown.

8. Patents ⇐104

Record of patent prosecution before Patent and Trademark Office (PTO) established substantial likelihood that someone at PTO actually considered, or that reasonable examiner would have considered, patentee's declaration allegedly containing material misrepresentation regarding prior art, in making final allowability determination, for purposes of inequitable conduct defense; although declaration was filed several months after examiner made initial allowability determination, there was circumstantial evidence that examiner subsequently considered declaration after notifying patentee that some of its claims had been rejected as obvious. 35 U.S.C.A. § 135; Practice Rules in Patent Cases, §§ 1.606, 1.641, as amended, 35 U.S.C.A.App.

9. Patents ⇐104

Patent examiner-in-chief had power to consider declarations submitted after initial determination of allowability as part of sua sponte patentability analysis before making final allowability determination. 35 U.S.C.A. § 135; Practice Rules in Patent Cases, § 1.641, as amended, 35 U.S.C.A.App.

10. Patents ⇐323.2(3)

Evidence in action for infringement of patent pertaining to immunoassay test for human immunodeficiency virus (HIV) raised genuine issue of material fact as to whether patentee submitted declaration to patent examiner that contained material misrepresentations regarding scope of prior art, precluding summary judgment on alleged infringer's inequitable conduct defense; declaration disclosed that patentee's project regarding hepatitis A virus had been unsuccessful, but failed to disclose successful projects regarding two other viruses. Fed.Rules Civ.Proc. Rule 56, 28 U.S.C.A.

11. Patents ⇐36(1)

Testimony of alleged prior inventor or inventors, standing alone, is insufficient to prove prior invention; it must be corroborated by other evidence. 35 U.S.C.A. § 102(g).

12. Patents ⇐36(1)

Alleged inventor's laboratory notebooks suffice for purposes of corroboration of claim of prior inventorship, even where they are not contemporaneously witnessed by inventor. 35 U.S.C.A. § 102(g).

13. Patents ⇐90(1)

"Conception," for purposes of priority of invention claim, is formulation of definite permanent idea of complete and operative invention, as it is hereafter to be applied in practice. 35 U.S.C.A. § 102(g).

See publication Words and Phrases for other judicial constructions and definitions.

14. Patents ⇐90(1)

In ordinary context, inventor need not demonstrate that invention actually works for conception to be complete; that discovery is part of reduction to practice, not conception. 35 U.S.C.A. § 102(g).

15. Patents ⇐90(1)

Immunoassay test for human immunodeficiency virus (HIV) was not conceived of by patentee for priority purposes when patentee had idea for recombinant immunoassay based on "env region" polypeptides; under doctrine of simultaneous conception and reduction to practice, knowledge of both nucleotide sequence of HIV fragment and operative method of isolating fragment was required. 35 U.S.C.A. § 102(g).

16. Patents ⇐90(5)

Act of filing patent application constitutes constructive reduction to practice of invention described therein. 35 U.S.C.A. § 102(g).

17. Patents ⇐90(5)

To constitute constructive reduction to practice, patent application must satisfy statutory disclosure requirements: it must set forth written description of invention, enable one of ordinary skill in the art to make a new invention, and set forth best mode for carrying out invention. 35 U.S.C.A. §§ 102(g), 112.

18. Patents ⇐323.2(3)

In action for infringement of patent pertaining to immunoassay test for human immunodeficiency virus (HIV), evidence raised genuine issue of material fact as to whether patentee's competitor successfully reduced invention to practice prior to patentee, precluding summary judgment on alleged infringer's priority defense; fact issues remained as to when patentee reduced invention to practice and whether competitor had completed required deoxyribonucleic acid (DNA) sequencing. 35 U.S.C.A. § 102(g).

19. Patents ⇐98

Patent application purporting to set forth recombinant human immunodeficiency virus (HIV) immunoassay did not meet statutory enablement requirement, and thus could not constitute constructive reduction to practice for purposes of alleged infringer's priority defense in action for infringement of patent pertaining to immunoassay test for HIV virus; application required starting materials that were neither described in application nor available in prior art, and relied on information about structure and nucleotide sequence of HIV that was neither disclosed in application or available in prior art. 35 U.S.C.A. §§ 102(g), 112.

20. Patents ⇐98

Best mode rule requires inventor to set forth in patent application best mode known

to applicant for practicing invention; purpose of this requirement is to prevent inventor from obtaining patent protection without actually having to set forth means for its successful practice. 35 U.S.C.A. § 112.

21. Patents ⇐98

Patent application purporting to set forth recombinant human immunodeficiency virus (HIV) immunoassay failed to satisfy statutory best mode requirement, and was not constructive reduction to practice for purposes of alleged infringer's priority defense in action for infringement of patent pertaining to immunoassay test for HIV; application did not set forth adequate information for obtaining or making necessary starting material. 35 U.S.C.A. § 112.

22. Patents ⇐90(5)

Invention relating to immunoassay test for human immunodeficiency virus (HIV) could not be conceived, for purposes of priority defense in infringement case, before it was actually reduced to practice.

Harold J. McElhinny, Michael A. Jacobs, Morrison & Foerster, San Francisco, CA, for Chiron Corp.

Curtis E.A. Karnow, Stephen C. Lewis, Landels Ripley & Diamond, San Francisco, CA, for Abbott Laboratories.

OPINION

PATEL, District Judge.

Plaintiff Chiron Corporation ("Chiron") brought this action against defendant Abbott Laboratories ("Abbott"), alleging infringement of U.S. Patent No. 5,156,949 ("'949 patent"), which pertains to an immunoassay test for the HIV virus. In its answer, Abbott alleges as two of its defenses inequitable conduct and prior invention. Now before the court are cross-motions for summary judgment on these two defenses.

Having considered the parties' arguments and submissions, and for the reasons set forth below, the court enters the following memorandum and order.[1]

BACKGROUND[2]

After the first documented cases of what is now commonly called Acquired Immune Deficiency Syndrome ("AIDS") occurred in the United States in 1981, researchers identified the Human Immunodeficiency Virus ("HIV") as its primary cause.[3] Antibody tests called "immunoassays" were developed thereafter, in order to detect the presence of antibodies to HIV in human blood and thus serve as a means of determining whether the blood is infected with the virus. This action arises out of the development and patenting of these immunoassays.

A. *General Background on HIV*

HIV is a virus,[4] and like other viruses it consists of genetic material and proteins, in the form of a string of nucleotides. Genetic material is the blueprint for all proteins and polypeptides (which are either full or partial

1. The court issued a tentative memorandum and order on May 31, 1995, and oral argument was held on that order on June 9, 1995. Per the court's instructions at that hearing, the parties have submitted supplemental briefing and made supplemental evidentiary submissions, all of which have been taken under submission along with the original briefing and evidence. The court has found that issuing a tentative order and having the parties focus on that order at oral argument and file supplemental briefing has been extremely useful, and is a practice worth repeating in similar cases. With issuance of this order, the court's tentative order is vacated and withdrawn.

2. All facts in this section are derived from the Joint Statement of Undisputed Facts submitted by the parties and other undisputed portions of the record. In its supplemental briefing on the tentative order, Chiron challenges a number of the facts recited in this section. Chiron's challenges must fail. The facts in this section are those to which Chiron stipulated in the joint statement, and it cannot now attempt to disavow facts to which it has stipulated.

3. In the early years of AIDS research, the virus was known by several acronyms, including the current HIV as well as HTLV-III and LAV, among others. For the sake of consistency and simplicity, the court employs the acronym HIV, except where otherwise necessitated by the record.

4. Actually, because its genetic material is RNA rather than DNA, HIV is a retrovirus.

proteins). Genetic material comes in two types: DNA and RNA. A gene is a set of nucleotides that contains the blueprint for a specific protein.

HIV's genetic material is RNA. Although HIV's RNA contains an entire blueprint for HIV, HIV lacks the ability to reproduce by itself. Thus, HIV must use the genetic material of a host cell (such as a human white blood cell) to reproduce itself. HIV enters the host cell, releases its RNA, and makes a DNA copy of its RNA. This DNA copy is then incorporated into and becomes part of the genetic material of the host cell, which begins to make copies of the HIV virus, spreading the infection and often killing the host cell.

When the human immune system detects the presence of HIV, it responds with HIV antibodies and defensive cells. Antibodies are unique molecules formed by the immune system in response to infection, and HIV-specific antibodies are formed as part of the immune system's defensive efforts. These antibodies bind to the HIV virus, and the presence of these antibodies in a human blood sample indicates a current or prior encounter with the HIV virus or part of the virus.

B. *Development of an HIV Immunoassay*

Immunoassays can be used to detect viral antibodies, and an HIV immunoassay thus can detect the presence of HIV in human blood. Detection is made possible by the reaction or binding that occurs between viral peptides and virus-specific antibodies, a process known as "immunoreactivity." The immunoassays used to detect HIV can be constructed with either natural polypeptides or recombinant polypeptides. An HIV immunoassay using natural polypeptides can be constructed by growing live, fully intact HIV in large quantities, breaking the HIV into pieces, collecting the proteins, sticking them to a surface, and then washing blood over the surface. If HIV antibodies are present in the blood, they bind to the proteins and remain attached to them when the blood is washed from the surface. To detect the HIV antibodies bound to the HIV proteins, enzymes that change color or fluorescent markers that emit light can be used to attach to the antibodies.

The problem with a natural polypeptide immunoassay is that it requires growing large amounts of live, intact HIV, which exposes workers to risk of infection and requires expensive laboratory facilities. Thus, as research began in 1984 on creating an HIV immunoassay, scientists sought to use recombinant DNA technology to make proteins and partial proteins from HIV's outer layer, called the envelope ("env"). Because scientists suspected that the env was immunoreactive, the focus in 1984 was to identify DNA fragments that encoded env polypeptides. In late Spring 1984, a number of teams were fast at work trying to develop such a test, including projects at Chiron, DuPont, and a collaborative project between the National Institutes of Health ("NIH") and Centocor ("NIH/Centocor"). All three teams attempted to create an env based recombinant DNA immunoassay.

To make an artificial env protein or polypeptide, large quantities of HIV genetic material are required. This material can be obtained by copying or cloning HIV's genetic material and making many DNA copies. As scientists decipher the genetic material, they display it on maps showing where genes are located in relation to one another in the material. In order to locate the env region of HIV, scientists from both the NIH and Chiron studied maps of other viruses that they believed would be structurally similar to HIV. Based on study of those viruses, the scientists determined that the genes for HIV's env likely would be in the right half of HIV's genetic material, and began looking for it there.

To locate and isolate particular regions of genetic material, scientists make "restriction maps" by cutting DNA into progressively shorter fragments through a series of reagents called "restriction enzymes." Restriction maps divide DNA into fragments that can be described and defined by "restriction sites." The restriction enzymes recognize specific sequences of nucleotides and cut genetic material at those sites. Different restriction enzymes recognize different sites, a

capability that makes it possible to isolate specific regions of genetic material accurately and repeatedly. Restriction sites are included on the restriction maps of the genetic material. While restriction maps enable scientists to identify and "cut" specific fragments of DNA, "sequencing" is more precise, and enables scientists to create a complete map of the exact order of the nucleotides comprising the DNA.

Between June 1984 and September 1984, the NIH ran sequencing reactions designed to map the nucleotide sequence of an HIV-clone called BH-8. The NIH collaborated with DuPont, Centocor, and Dana-Farber to map the nucleotide sequence of HIV DNA fragments in BH-8, as well as two other HIV clones, BH-10 and BH-5. DuPont received clone BH-10 from Dr. Flossie Wong-Staal of the NIH on or about July 6, 1984, and began generating nucleotide sequence data from BH-10 on or about July 18, 1984. By September 1, 1984, DuPont had run sequence reactions on DNA fragments that spanned what has now been determined to be the entire env region of BH-10's DNA. DuPont obtained U.S. Patent No. 4,861,707, issued in 1989 based on a February 1987 application, which states that a BglII-BamHI BH-10 DNA fragment encodes an immunoreactive polypeptide from the env region.

The NIH gave the BH-10 and BH-8 clones to Dr. Nancy Chang at Centocor on July 20, 1984, and also gave Centocor a restriction map for BH-10, BH-8, and BH-5. At some point after July 20, 1984, Dr. Chang and her colleagues started working on expressing recombinant env proteins through random and directed cloning. On or about July 26, 1984, Dr. Chang met with Dr. Scott Putney, an outside scientist who was working with Centocor on its HIV research, to plan Centocor's HIV clone research. A July 31, 1984 laboratory notebook entry by one Centocor scientist is entitled "Sequencing of SstI-HindIII fragment." This fragment—SstI-HindIII—is a BH-8 fragment that contains approximately 76% of the env region of BH-8. Dr. Chang drafted a statement, dated August 27, 1984, stating that Centocor had "sequenced a segment (about 8,500 base pairs long) of [HIV's DNA] encoding most of the *env* gene."

On October 10, 1984, Centocor filed U.S. Patent Application No. 659,339 ("'339 application"), which is still pending. The '339 application purports to set forth, *inter alia*, an env based recombinant HIV immunoassay, and lists the following expression vectors: "OmpA, pIN (A, B, and C), lambda pL, T7, lac, Trp, ORF and lambda gt11." It does not identify the HIV clones upon which it relies as starting material. The '339 application lists only Dr. Chang as an inventor, and lists Centocor as the assignee. It does not indicate that Dr. Chang deposited any of the HIV clones with the American Type Culture Collection ("ATCC") [5] on or before October 10, 1984, or refer to any ATCC deposit relating to HIV clones. On or about August 22, 1984, Dr. Wong-Staal, Dr. Robert Gallo, Dr. Beatrice Hahn, and a fourth NIH scientist deposited BH-10 and BH-8 with the ATCC, and filed U.S. Patent Application No. 643,306 ("'306 application"), purporting to set forth the process for cloning the complete HIV genome.

On August 9, 1984, Dr. Hahn and others submitted an article to the journal *Nature* that was published on November 8, 1984 as *Molecular Cloning and Characterization of the HTLV-III Virus Associated with AIDS*, 312 Nature 166 (1984). The article contains the first description in a scientific journal of BH-10, BH-8, and BH-5. On November 29, 1984, Dr. Chang, Dr. Wong-Staal and others submitted an article to the journal *Nature* which was published on January 24, 1985 as *Complete Nucleotide Sequence of the AIDS Virus, HTLV-III*, 313 Nature 277 (1985). On December 21, 1984, Dr. Chang and others submitted an article to the journal *Science* called *Expression in Escherichia of Open Reading Frame Gene Segments of HTLV-III*, which was published in April 1985. That article states that polypeptides produced from "fragments of HTLV-III DNA derived from BH-10" (numbers 127, 121 and 113)

5. The ATCC is a depository for living material, and deposition there is necessary for carrying out claimed inventions involving living material.

were immunoreactive. Figure 8 of the '339 application is a printout showing 3112 nucleotides from the HIV genome. Of the 3112 nucleotides there displayed, 883 fall within the env region of HIV, though the env nucleotides are not identified on the figure as such. The "Best Mode" section of the '339 application contains three separate embodiments of a recombinant HIV immunoassay. The second embodiment specifically discusses as examples the random breakage of three HIV DNA fragments—EcoRI–EcoRI, KpnI–KpnI, and EcoRI–HindIII—into 200–500 base pairs. The '339 application does not describe a method for obtaining HIV clones from the native virus.

On January 23, 1985, Centocor filed U.S. Patent Application No. 693,866 (" '866 application") with the PTO. The '866 application purports to set forth the complete nucleotide sequence of BH–10, BH–8, and BH–5. The '866 application lists only Dr. Chang as an inventor, and names Centocor as the assignee.[6] Clones 113, 121 and 127 described in the '866 application are from the env region, but not from the portion of the HIV env region for which sequence information was included in Figure 3 of the '339 application.

By October 1, 1984, Chiron had run sequence reactions on DNA fragments that spanned what has since been determined to be the entire env of HIV. On October 31, 1984, Chiron filed U.S. Patent Application No. 06/667,501, the ancestral patent application that subsequently led to the '949 patent, which was issued on October 22, 1992 to Chiron.[7] The '949 patent teaches that DNA fragments as short as 21 nucleotides can be used to generate immunoreactive polypeptides from HIV's env.

C. *The Steimer Declaration*

During the pendency of the application that later became the '949 patent, Chiron scientist Dr. Kathelyn Sue Steimer submitted a Rule 132 Declaration to the PTO, which she signed on September 14, 1990. The version of this declaration in the official PTO file does not have a mailroom stamp or other date stamp indicating when it was received at the PTO. However, the declaration was submitted with a Supplemental Response, which is date-stamped as having been received by the Board of Patent Appeals and Interferences on September 20, 1990. In her declaration, Dr. Steimer states that her expert opinion was that "the state of the art in October 1984 was at best speculative with respect to the general usefulness of recombinant antigens in immunoassays." She based this conclusion on a number of factors, including (1) Chiron's failure to develop a similar recombinant immunoassay for use with Hepatitis A virus (HAV), and (2) the fact that naturally derived HIV DNA varies greatly due to transcription error, while recombinant DNA does not, with the result that recombinant based immunoassays might not succeed in binding to actual HIV antibodies. Based on this assessment, Dr. Steimer made the critical conclusion that, "in October 1984 those of even greater than ordinary skill in the art could not have had any reasonable expectation that a HIV diagnostic using a recombinant antigen would have been as effective as the 'native' HIV diagnostic then known."

In a submission filed with the government in May 1984, Chiron stated that it "is developing vaccines to protect cats against infection by the retrovirus FeLV. We believe that these efforts will have great significance in understanding, diagnosing, and perhaps treating and preventing AIDS since some of the fundamental pathological features are shared by FeLV and the retrovirus associated with AIDS." In April 1986, Chiron filed a patent application claiming, in part, "[a] particle immunogenic against HAV infection which particle comprises a polypeptide having an amino acid sequence capable of forming a particle when said sequence is produced in a eucaryotic host, and an epitope of

6. In May 1986, Centocor asked the PTO to amend the inventorship of the '866 application to include Dr. Gallo and Dr. Wong-Staal.

7. A series of patent applications led to the '949 patent. The history of the patent prosecution is as follows: (1) Application No. 06/667,501 filed 10/31/84, abandoned 7/14/86; (2) Application No. 06/696,534 filed 1/30/85, abandoned 7/14/86; (3) Application No. 06/773,447 filed 9/6/85, abandoned 9/12/88; (4) Application No. 07/138,894 filed 12/24/87, granted as '949 patent.

HAV." This patent application is for an HAV vaccine, not an HAV assay or diagnostic, and does not mention the use of recombinant HAV polypeptides in a diagnostic.

On July 2, 1990, Examiner Christine Nucker forwarded a PTO Form 850 to the Board of Patent Appeals and Interferences in Interference No. 102,432. This interference involved a potential conflict between claims in the pending Chiron patent application (Application No. 07/138,894, which ultimately issued as the '949 patent and which was the subject of the Steimer Declaration), and a patent application by Essex et al. (Application No. 07/539,370). On the Form 850, Nucker indicated that there were allowable claims in the pending application that were in potential conflict with the Essex application, necessitating an interference. Specifically, under the section entitled "The claims of this party which correspond to this count," Nucker wrote "60–81 (allowable)." These are the claims that ultimately appeared as claims 1–22 of the '949 patent as issued.

In her March 1992 Notice of Allowability for the '949 patent, Examiner Nucker listed the communications from Chiron upon which she relied and to which she was responding. Dr. Steimer's declaration is not listed as such a communication. Other than the final Notice of Allowability, there is no evidence in the record that the PTO took any action subsequent to Dr. Steimer's declaration.

The invention described in the '949 patent is an immunoassay reactive with human AIDS sera based on recombinant DNA proteins from the env region of HIV.

LEGAL STANDARD

Under Federal Rule of Civil Procedure 56, summary judgment shall be granted:

> against a party who fails to make a showing sufficient to establish the existence of an element essential to that party's case, and on which that party will bear the burden of proof at trial ... since a complete failure of proof concerning an essential element of the nonmoving party's case necessarily renders all other facts immaterial.

Celotex Corp. v. Catrett, 477 U.S. 317, 322–23, 106 S.Ct. 2548, 2552, 91 L.Ed.2d 265 (1986). The party moving for summary judgment has the "initial responsibility of informing the district court of the basis for its motion, and identifying those portions" of the record showing the absence of a genuine issue of fact. *Id.* at 323, 106 S.Ct. at 2553. The burden then shifts to the nonmoving party to present evidence sufficient to support a verdict in its favor on every element of its claim for which it will carry the burden of proof at trial. *Id.* at 322–23, 106 S.Ct. at 2552. "If the [nonmoving party's] evidence is ... not sufficiently probative ... summary judgment may be granted." *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 249–50, 106 S.Ct. 2505, 2511, 91 L.Ed.2d 202 (1986).

[1, 2] Where the moving party has the burden of proof on a claim or defense raised in a summary judgment motion, it must show that the undisputed facts establish every element of the claim or defense. *Meyers v. Brooks Shoe Inc.*, 912 F.2d 1459 (Fed.Cir. 1990). When the defendant in a patent infringement case moves for summary judgment on an affirmative defense, the elements of which the defendant must prove by clear and convincing evidence, the non-moving party must simply produce enough evidence to allow a rational trier of fact to find that there is not clear and convincing evidence. As a result of this unusual posture, the non-moving party's burden to come forward with evidence to prevent summary judgment is less stringent than that normally placed on a non-moving party. *Schneider (USA) Inc. v. C.R. Bard Inc.*, 18 U.S.P.Q.2d 1076, 1080, 1990 WL 292143 (D.Mass.1990).

[3] The court's function on a motion for summary judgment is not to make credibility determinations, *Anderson*, 477 U.S. at 249, 106 S.Ct. at 2510, and the inferences to be drawn from the facts must be viewed in a light most favorable to the party opposing the motion. *T.W. Elec. Serv. v. Pacific Elec. Contractors Ass'n*, 809 F.2d 626, 631 (9th Cir.1987).

DISCUSSION

[4] Chiron's patent is presumed by statute to be valid. 35 U.S.C. § 282. According-

ly, in order to prevail on its inequitable conduct and prior invention defenses, Abbott must prove those defenses by clear and convincing evidence. *Avia Group Int'l, Inc. v. L.A. Gear California, Inc.*, 853 F.2d 1557, 1562 (Fed.Cir.1988).

I. *Inequitable Conduct*

Abbott bases its inequitable conduct defense solely on Chiron's submission of the Steimer Declaration to the PTO in conjunction with Patent Application No. 07/138,894. Abbott argues that the Steimer Declaration contains material misrepresentations and omissions, and that it was intended to deceive the PTO. Specifically, Abbott contends that the declaration misrepresents the state of the art in 1984 by concluding that at that time, there could be no reasonable expectation that a recombinant DNA based immunoassay could be effective, and that it fails to mention previous work done by Chiron itself with recombinant DNA immunoassays for other viruses. Abbott also argues that it is irrelevant to the inequitable conduct defense whether the Steimer Declaration actually was received and considered by the PTO in making its allowability determination.

In response, Chiron argues first that the Steimer Declaration cannot form the basis of an inequitable conduct defense because it was submitted months after the claims had already been found allowable by the PTO. In the alternative, Chiron argues that the declaration does not contain material misrepresentations or omissions, and even if it does, there is insufficient evidence of intent.

A. *Analytical Framework*

[5] The party asserting an inequitable conduct defense bears the burden of proving both intent and materiality by clear and convincing evidence. *Braun Inc. v. Dynamics Corp. of America*, 975 F.2d 815, 822 (Fed.Cir. 1992); *see also Halliburton Co. v. Schlumberger Technology Corp.*, 925 F.2d 1435, 1443–44 (Fed.Cir.1991). Accordingly, Abbott must prove by clear and convincing evidence that the Steimer Declaration contained material misrepresentations or omissions and that Dr. Steimer intended to deceive the PTO.

[6, 7] Information is material if "there is a substantial likelihood that a reasonable examiner would consider [the information] important in deciding whether to allow the application to issue as a patent." 37 C.F.R. § 1.56(a); *Halliburton*, 925 F.2d at 1440. A finding of materiality alone, however, is insufficient to support a finding of an intent to deceive, which is a separate element of inequitable conduct. Thus, in attempting to prove inequitable conduct, the accuser may not rely solely on the materiality of information allegedly withheld. *Braun*, 975 F.2d at 822. To support a finding of inequitable conduct, the "involved conduct, viewed in light of all the evidence, including evidence indicative of good faith, must indicate sufficient culpability to require a finding of intent to deceive." *Kingsdown Medical Consultants, Ltd. v. Hollister, Inc.*, 863 F.2d 867, 876 (Fed.Cir.1988), *cert. denied*, 490 U.S. 1067, 109 S.Ct. 2068, 104 L.Ed.2d 633 (1989). Gross negligence is not in and of itself sufficient to establish inequitable conduct; rather, deceitful intent must be shown. *Halliburton*, 925 F.2d at 1442–43; *Kingsdown*, 863 F.2d at 876; *see also Hewlett–Packard Co. v. Bausch & Lomb Inc.*, 882 F.2d 1556, 1562 (Fed.Cir.1989) (reckless indifference to the truth insufficient to show intent), *cert. denied*, 493 U.S. 1076, 110 S.Ct. 1125, 107 L.Ed.2d 1031 (1990).

B. *Analysis*

[8] At the threshold, the court must determine whether the PTO actually considered the Steimer Declaration in deciding to grant the '949 patent. As Chiron points out, the Steimer Declaration was filed several months after Examiner Nucker made an initial determination of allowability, and other than the issuing of the final notice of allowability in March 1992, there is no direct evidence that the PTO took any action in response to (or otherwise considered) the declaration. In addition, Chiron has submitted two declarations from its putative expert stating that in his opinion, the PTO did not consider the Steimer Declaration. Goolkasian Decl. ¶ 12; Goolkasian Supp.Decl. ¶ 7. However, as Abbott argues, it would have been proper for the PTO to consider the declaration subsequent to the initial allowability determina-

tion, and there is circumstantial evidence that such consideration likely occurred.

Chiron is correct that Nucker made at least an initial finding of allowability of the claims in the '949 patent no later than July 2, 1990, when she forwarded the Form 850 to the Board of Patent Appeals and Interferences ("BPAI") based on claims 60–81 in the pending application.[8] Chiron also is correct that, pursuant to PTO rules, before Nucker could have sent the Form 850 to the BPAI, she first had to determine that the claims listed therein were otherwise patentable to Chiron. *See* Manual of Patent Examining Procedure ("MPEP") §§ 2305, 2306, 2307.02 (5th ed. 1994); *see also* 37 C.F.R. § 1.606 ("Before an interference is declared between an application and an unexpired patent, an examiner must determine that there is interfering subject matter claimed in the application and the patent which is patentable to the applicant subject to a judgment in the interference.").

[9] However, this initial determination is not necessarily final, *see* MPEP at § 2341; 37 C.F.R. § 1.641, and the examiner-in-chief had the power to consider the Steimer Declaration as part of a *sua sponte* patentability analysis before making a final allowability determination, 35 U.S.C. § 135. Furthermore, there is evidence in the record, albeit circumstantial, that the PTO may actually have considered the Steimer Declaration subsequent to the filing of the Form 850. Specifically, the following circumstantial evidence is relevant to Abbott's allegation. On July 19, 1989, Examiner Nucker notified Chiron that she had rejected some of the claims in the application on the basis of obviousness. In direct response to that notice, Claims 60–81 were added by Chiron on January 19, 1990. Subsequently, on February 14, 1990, the PTO held an interview with a Chiron representative, at which Examiner Nucker was present. The report of that interview states that all claims were discussed, and that agreement was *not* reached on the issues discussed. The report sets forth two issues that apparently were discussed, one of which was: "diff. between lysate and recombinant assay (heterogeneity from innoculate (diff. isolates) + mutations in culture) due to hypervariation regions—recombinant homogenous antigen."[9]

In late September 1990, Chiron filed its Supplemental Response with the PTO, which included the Steimer Declaration, an amendment of inventorship, and a supplemental list of references. The submission purported to be in further response to Nucker's July 19, 1989 action, though it also referenced the February 1990 interview.[10] In her declaration, Dr. Steimer directly addressed the problem cited in the report of the February 1990 interview referenced above—the difference between a lysate (native) produced and a recombinant produced assay due to the heterogeneity of the native antigen (resulting from natural variations in the amino acid sequence) and the necessarily homogeneous nature of the recombinantly produced antigen. Steimer Decl. ¶ 8. Thus, it appears that the Steimer Declaration responded to questions raised by Nucker in the February 1990 interview that had, subsequent to the interview, not been answered any other way.[11]

8. It is undisputed that these claims subsequently became claims 1–22 in the '949 patent as issued.

9. Chiron expends considerable effort arguing that the issues listed on the interview report were *not* those upon which agreement was not reached, but only those issues that were discussed. While this interpretation of the report is plausible, it is not obvious on the face of the document that this necessarily is the case, and the fact that Steimer subsequently submitted a Rule 132 declaration discussing the key issue listed there certainly is evidence that the issue had yet to be resolved.

10. Chiron references a February *20*, 1990 interview with the examiner, but it appears that it was referring to the February *14*, 1990 interview, the notice of which was mailed on February 20.

11. In its supplemental citations, Chiron points to two previous submissions to the PTO that addressed this problem. *See* Goolkasian Supp. Decl.Ex. A (Haigwood Decl.) ¶ 5, Ex. D (Chiron's January 1990 Amendment) at 9–17. However, each of these documents was submitted *prior* to the February 1990 interview, after which the issue apparently remained open notwithstanding those submissions. Indeed, Chiron itself concedes that the Steimer Declaration "provided additional citations to scientific articles discussing the heterogeneity of HIV, while reiterating the same point made by Dr. Haigwood and in

The formal referral to the BPAI took place on September 28, 1990, subsequent to submission of the Steimer Declaration (and more than two months after Nucker filled out the Form 850). The formal reference indicates that claims 60–81 of the Chiron application were in potential conflict with the Essex application.

It is clear that on interference, the BPAI may consider patentability and may recommend rejection of a claim even if it is not involved in the interference. 37 C.F.R. §§ 1.655, 1.659; MPEP §§ 2314, 2355, 2359. The examiner-in-chief, who is responsible for declaring the interference, and the BPAI, have the entire application file. 37 C.F.R. § 1.609; MPEP § 2311. Furthermore, until the interference is declared the examiner continues to have jurisdiction over the pending application. 37 C.F.R. § 1.614(C); MPEP § 2314. In this case the interference was declared on or shortly before September 28, 1990. Until that time, Examiner Nucker (or another examiner) continued to have jurisdiction.

The Initial Memorandum so heavily relied upon by Chiron's expert bears the handwritten legend at the top: "Ready for Declaration—MLC Caroff—(9/28/90)" and the number "102432", which is the case number for this interference. Goolkasian Decl.Ex. 2. This legend is not explained on the document or by the parties. It may relate to the Notice of Declaration required by 37 C.F.R. § 1.611 and MPEP § 2311. In any event, Chiron's supplemental response, which included the Steimer Declaration, is stamped "Received" by the BPAI on September 21, 1990, seven days *before* Examiner-in-Chief Caroff declared the interference pursuant to section 1.610 of the regulations and MPEP § 2311. At any of these stages, the examiner, the examiner-in-chief, and the BPAI would have had the Steimer Declaration and likely would have considered it since it specifically addressed issues that remained outstanding.

The BPAI's ruling in favor of Chiron's claims came down on September 27, 1991, and the formal notice of allowability on claims 60–81 went out on March 20, 1992. In that notice, Examiner Nucker stated as her reason for allowability that:

> applicants were the first to realize the importance of utilizing recombinantly produced HIV envelope proteins as antigen standards in immunoassays for anti-HIV antibodies. By generating the envelope proteins recombinantly it is possible to control for the high rate of mutation seen in these proteins when produced by virally infected cells. This control results in improved assay results when compared with extant commercially available kits.

This language appears to relate to the questions raised in the February 1990 interview and the answers provided, in part, by Dr. Steimer in her declaration. A reasonable factfinder therefore could infer that despite Examiner Nucker's failure to list the Steimer Declaration as one of the Chiron documents upon which she relied and to which she was responding, she, another examiner, the examiner-in-chief, or the BPAI actually had considered the content of the Steimer Declaration in making a final determination. Absent such an inference, the record contains no post-interview evidence that Nucker's critical questions regarding the heterogeneity/homogeneity problem ever were answered. That problem was of sufficient import to prompt the February 1990 inquiry, the Steimer Declaration responded to it, and the March 20, 1992 allowance specifically referenced it.[12]

Accordingly, based on the record of the patent prosecution before the PTO, there is a substantial likelihood, and a reasonable factfinder could conclude, that someone at the

Chiron's January 1990 Amendment." Chiron's Additional Citations at ¶ 14. Chiron offers no explanation why further citations and reiteration was necessary after the interview if the issue already was resolved. These prior submissions do not alter the fact that other than the Steimer Declaration, nothing in the post-interview record relates directly to the critical issue.

12. Furthermore, a second notice went out on April 27, 1992, correcting the inventorship as a result of the September 21, 1990 submissions. The fact that Nucker considered the amended notice of inventorship in the September 1990 supplemental response further raises the possibility that she also considered the Steimer Declaration, which was part of the same submission.

PTO actually considered, or that a reasonable examiner in such circumstances would have considered, the Steimer Declaration in making the final allowability determination. Thus, the court must proceed to analyze whether there is sufficient evidence from which a reasonable factfinder could (or must) find by clear and convincing evidence that the Steimer Declaration evidences both materiality and intent within the meaning of inequitable conduct.

[10] Abbott cites four allegedly material misrepresentations/omissions in the Steimer Declaration, and the court will examine each in turn.

1. *Status of the HAV Project*

Abbott first points to Steimer's discussion of the status of Chiron's HAV project, which she characterized as a "complete failure" because "virtually none" of the recombinant antigens bound with antibodies to the virus. Steimer Decl. ¶¶ 6–7. Steimer also stated that the HAV project ultimately was abandoned because of the unpredictable results from the recombinant HAV immunoassay. *Id.* Abbott argues that these statements constitute a material misrepresentation because Steimer stated that the HAV project failed *in 1984*, when the project in fact was not abandoned until two years later, such that its failure was not relevant to the state of the art in 1984. Abbott also contends that in fact, the state of the art in 1984 was a belief that recombinant antigens would work.

Abbott's reliance on Steimer's statements regarding the HAV project is misplaced. Dr. Steimer did not represent in her declaration that the HAV project failed *in 1984*; instead, she stated that in 1984 the HAV virus was better characterized than HIV, and that the HAV project nonetheless failed and ultimately was abandoned. Steimer Decl. ¶¶ 6–7. She also couched her statement in the present tense: she stated that "a case which illustrates that producing a successful recombinant immunoassay *is* not merely a hypothetical problem *is* shown by Chiron's work with [HAV]." *Id.* at ¶ 6 (emphasis supplied). Nothing in this language implies that the HAV project already had failed or been abandoned in 1984.

The only reasonable reading of the Steimer Declaration is that in 1984, the HAV virus was sufficiently well understood to justify an attempt at creating a recombinant based immunoassay, that that attempt *ultimately* failed, and that such failure illustrates that successful use of recombinant based immunoassays therefore is not now, and was not in 1984, guaranteed. When the language in the declaration is viewed in the context of the entire declaration, no rational factfinder could conclude that this statement is a material misrepresentation of fact.[13]

Furthermore, Abbott's argument that in 1984 the state of the art was a belief that recombinant antigens would work is not in fact contradicted by the Steimer Declaration. Dr. Steimer concluded only that "the state of the art in October, 1984 was *at best speculative* with respect to the general usefulness of recombinant antigens in immunoassays." Steimer Decl. ¶ 5. There is nothing in any of the evidence submitted by either party that indicates that this (fairly equivocal) conclusion was inaccurate. Indeed, far from calling Steimer's statement into question, Abbott's emphasis on the fact that Chiron did not even express recombinant proteins from HAV until 1985 *bolsters* the statement's accuracy.

No reasonable factfinder could conclude that Steimer's statement regarding the status of the HAV work constituted a material misrepresentation, and Abbott cannot rely on the HAV work to support its inequitable conduct defense.

2. *Omission of the HBV Project*

Abbott next argues that Steimer selectively excluded successful work Chiron had done prior to October 1984 with the HBV virus. Specifically, Abbott contends that Steimer

13. In a declaration filed with the PTO as part of the Essex interference, Chiron scientist Stella Quan explained that the HAV project failed in the period 1985–1987. Goolkasian Supp. Decl.Ex.B. This evidence of good faith would make it impossible for Abbott to demonstrate by clear and convincing evidence that Chiron had the necessary intent to deceive the PTO as to the HAV project.

failed to mention that Chiron had created an HBV diagnostic kit by October 1984, and contends that the work with HBV was far more relevant to the HIV project than the failed HAV project, and that its omission was material.

Abbott provides sufficient evidence of a material omission to survive, though not prevail upon, summary judgment with respect to the HBV work.

Abbott has submitted undisputed evidence that by 1984 Chiron had successfully created a recombinant based HBV diagnostic kit, and that Chiron represented in a project application with the U.S. Department of Health and Human Services in 1984 that an HIV immunoassay possibly could follow on that model. Abbott Ex. 2U at 1205775; Dina Depo. at 424–27. While there is no evidence in the record that the work with HBV was somehow *more* relevant to the HIV work than the failed HAV project,[14] certainly Chiron thought it sufficiently relevant to the HIV work to make that representation part of an application for a government HIV project, Abbott Ex. 2U at 1205775, and its success in creating a recombinant based immunoassay for HBV stands in fairly stark contrast to the failed HAV project which Steimer chose to report to the PTO.[15]

The court finds it difficult on the current record to understand how the HBV work could be relevant to Chiron's government HIV project application and at the same time not be material to the patent application, particularly to Steimer's declaration. A reasonable factfinder could conclude by clear and convincing evidence that Steimer's failure to report the successful HBV project constituted a material omission, and further

14. Chiron's putative expert states only that in his opinion the HBV and FeLV projects were not *more* relevant than the HAV project, and that they would have been cumulative. Goolkasian Supp.Decl. ¶ 30. Nowhere does he state, however, that such work was irrelevant, or that reporting the HAV failure without the HBV success gave an accurate impression of the state of the art in 1984.

15. Chiron's reliance on the fact that it had not developed a *commercial* HBV kit is irrelevant, as the issue is not the marketing of such a kit but the success of the HBV project and its relevance to the HIV work. Furthermore, Chiron's reliance on the fact that its HBV project was based on "core" proteins, not "surface" proteins like those sought in the HIV work also is irrelevant. The point is not that the HBV project was identical to the HIV work, but that Chiron had successfully produced recombinant HBV proteins, that it represented that success to the government as relevant to the HIV work, and that it failed to report that success to the PTO. Furthermore, Dr. Luciw testified that Chiron was working with surface HBV antigens as well, though he could not recall the specifics, and that it was this work that led him to believe that a recombinant HIV immunoassay would work. Luciw Depo. at 142–44, 146. Chiron also erroneously relies on the fact that there is no evidence from its HBV project that a recombinant antigen would be "as effective" as a natural one. Again, this is irrelevant. The omission of the HBV project does not become non-material simply because that project does not show that recombinant assays are as effective as native assays. Its materiality is based on the fact that Chiron had successfully produced recombinant HBV proteins for use in an assay, and believed itself (and represented to the government) that such success was relevant to the HIV work.

Finally, Chiron's contention that Dr. Steimer was personally unaware of the HBV work at the time of her declaration is disingenuous. Citing Dr. Steimer's deposition testimony, Chiron argues that it is undisputed that Steimer "had no knowledge of Chiron's HBV work." Chiron's Further Submission Re: Inequitable Conduct at 6 (citing Steimer Depo. at 513–14). Careful review of Steimer's deposition reveals that Chiron has mischaracterized her testimony. Steimer was not asked, and did not testify, that she had *no* knowledge of Chiron's HBV work. In fact, the following colloquy took place:

> Q: ... [a]re you aware of any hepatitis B recombinant-based immunoassay, whether it involves surface antigens ... or some other recombinant proteins?
>
> A: It is possible that—and I don't know exactly where this effort is—but I was trying to recall after our discussion yesterday what it was I knew about hepatitis B diagnostics at Chiron, and I recalled that, in fact, I believe there is a need to—that that looking for antibodies to core is considered an important component of diagnostic diagnosis of chronic hepatitis B. *And I recall that Chiron was doing some work trying to develop hepatitis B core antigens, core antibody assay.* And I am not positive whether we were trying to use recombinant antigen in that format or not. That's really all I can recall at this time....

Steimer Depo. at 514. Thus, while it is clear Steimer was not necessarily aware of all of the details of the HBV work, she has admitted to being aware that such work was going on at Chiron, and a reasonable factfinder could conclude that her failure to report it to the PTO was purposefully misleading.

could infer that by referencing the failed HAV project but not the successful HBV work, Chiron intended to deceive the examiner as to the state of the art in October 1984. Accordingly, Abbott is entitled to rely on Chiron's omission of the HBV work to support its inequitable conduct defense, though it is not itself entitled to prevail upon that defense as a matter of law.

3. *Omission of the FeLV Project*

Abbott next argues that Steimer selectively excluded successful work Chiron had done prior to October 1984 with the FeLV virus. Specifically, Abbott contends that Steimer failed to mention that Chiron had successfully expressed immunoreactive env proteins from FeLV prior to October 1984, and contends that the work with FeLV was far more relevant to the HIV project than the failed HAV project, and that its omission therefore was material.

Abbott provides sufficient evidence of a material omission to survive, though not prevail upon, summary judgment with respect to the FeLV work.

While Chiron is correct that the evidence in the record reveals that its work with FeLV was aimed at creating a *vaccine*, not an immunoassay, Abbott Ex. 2U at 1205774–75, it also is undisputed that as part of that work Chiron created recombinant FeLV env antigens and determined their reactivity with antibodies to the virus. Luciw Depo. at 220–23; Abbott Ex. 2U at 1205775; Abbott Ex. 8P at 1230417.[16] There also is evidence in the record that because FeLV and HIV are both retroviruses, they have many similarities, and as a result Chiron's own scientists believed that the FeLV project was *more* relevant to the HIV work than their work with other viruses. Dina Depo. at 480. Attempting to justify its omission of the FeLV work from the Steimer Declaration, Chiron relies on the fact that the FeLV recombinant vaccine ultimately failed. Dina Depo. at 237–39. However, Chiron is unable to produce any evidence of the date the FeLV project failed (Dr. Dina testified only that it was "sometime" after September 1984), and the ultimate failure of the vaccine itself does not alter the very relevant fact that as part of its FeLV project, Chiron *was* successful in expressing recombinant env FeLV proteins that were immunoreactive with the virus. This fact alone makes the FeLV work directly relevant to the specific issues addressed in the Steimer Declaration (i.e. the efficacy of a recombinant immunoassay).[17]

A reasonable factfinder could conclude by clear and convincing evidence that Steimer's failure to report the successful FeLV project constituted a material omission, and further could infer that by referencing the failed HAV project but not the successful FeLV work, Chiron intended to deceive the examiner as to the state of the art in October 1984. Accordingly, Abbott is entitled to rely on Chiron's omission of the FeLV project to support its inequitable conduct defense, though it is not itself entitled to prevail upon that defense as a matter of law.

4. *Paragraph 4 of the Steimer Declaration*

Abbott finally argues that Dr. Steimer's expert opinion that "in October 1984 those of even greater than ordinary skill in the art could not have had any reasonable expectation that a HIV diagnostic using a recombinant antigen would be as effective as the 'native' HIV diagnostic then known," Steimer Decl. ¶ 4, is absolutely indefensible. Specifi-

16. In its tentative order, the court based its conclusion that omission of the FeLV work was not material on the fact that the FeLV work involved a vaccine, not an immunoassay. However, as Abbott's supplemental briefing and evidentiary submissions make clear, that fact is not dispositive. The evidence reveals that in its attempt to create a vaccine, Chiron created recombinant FeLV env antigens and determined their reactivity with antibodies to the virus—a process essentially identical to that employed in creating a recombinant immunoassay. Thus, the court is persuaded that its conclusion regarding the FeLV work in the tentative order was erroneous.

17. Chiron again erroneously relies on the fact that there is no evidence from its FeLV project that a recombinant antigen would be "as effective" as a natural one. Again, this is irrelevant. As with the HBV project, the omission of the FeLV project does not become non-material simply because that project does not show that recombinant assays are as effective as native assays. Its materiality is based on the fact that Chiron had successfully produced recombinant FeLV proteins that proved immunoreactive, and believed itself (and represented to the government) that such success was relevant to the HIV work.

cally, Abbott argues that in 1988 and 1984, Chiron's own scientist, Dr. Luciw, had a "high level of expectation" that the recombinant HIV diagnostic would work, Luciw Depo. at 148–49, such that the invention would not overcome the examiner's obviousness rejection. Abbott also points to Steimer's own admission (and Dr. Dina's concurrence) that her opinion was "too strong," Steimer Depo. at 167–69, 211–12; Dina Depo. at 562–63, and that instead the truth was that in 1984 there would be no reasonable "certainty" that it would work. Steimer Depo. at 168–69; Dina Depo. at 562–68. Abbott argues that a "lack of certainty" is materially different from "no reasonable expectation," and that the properly qualified opinion would not have overcome an obviousness rejection.

In response to this evidence, Chiron argues first that Dr. Steimer's feelings now regarding the accuracy of her declaration in 1990 are irrelevant, especially in light of the fact that she also has testified that at the time of the declaration, she believed in its fundamental accuracy. Steimer Depo. at 171, 204. Chiron also argues that Dr. Luciw did not testify that he had a high expectation of success that the recombinant HIV diagnostic would work *as well as* a native assay, but that he had a high expectation of achieving "demonstration of AIDS virus recombinant antigen reacting with antibodies from an infected host." Luciw Depo. at 149. Chiron argues that this testimony must be considered in the context of Luciw's further testimony that he agrees with the statement in paragraph 4. Luciw Depo. at 175, 179. Chiron also contends that it made it clear to the PTO that Steimer was not claiming that no one could have expected recombinant assays to have some usefulness, just that no one could have expected them to be as or more useful than native assays. *See* Chiron's Sept. 1990 Supp.Response at 4. Finally, Chiron's putative expert states that in his opinion, paragraph 4 was not intended to, and did not in fact, deceive the PTO. Goolkasian Decl. ¶ 15.

Chiron cites no authority for the proposition that its own scientists' admissions that statements made before the PTO were too strong are somehow irrelevant. Indeed, these admissions are perhaps the most damning evidence against Chiron. Chiron is correct that Dr. Luciw's testimony does not directly contradict paragraph 4 of the Steimer Declaration, and that Chiron was careful in its 1990 submission to caution the PTO that it was not claiming that there could have been no expectation that recombinant assays would be useful. *See* Chiron's Sept. 1990 Supp. Response at 4 ("Applicants are not arguing that some minimal degree of antigenicity was unpredictable. Rather, it is applicants' position that the performance of the recombinant env-based immunoassays '. . . is unexpectedly high in that it was as good or better than nonrecombinant assays."). However, while this language constitutes evidence of good faith for a factfinder to consider in its intent analysis, it does not go to materiality, and cannot alone overcome Dr. Steimer's own admission (concurred in by Dr. Dina) that her statement in paragraph 4 was too strong. Accordingly, notwithstanding Chiron's expert testimony, a reasonable factfinder could conclude that Steimer's opinion on this specific subject was material in that it enabled Chiron to overcome an obviousness rejection.

However, while there is sufficient evidence from which a reasonable factfinder could conclude by clear and convincing evidence that Steimer's statement in paragraph 4 is material, there is insufficient evidence of intent for Abbott to survive past summary judgment. The only evidence in the record that Chiron intended to deceive the PTO is Steimer's testimony that the exact wording of the paragraph, including the critical language "any reasonable expectation," was drafted for her by a lawyer, upon whom she relied to "use the appropriate language," Steimer Depo. at 169, and the fact that a misleading declaration was submitted in response to questions raised by the examiner.

In response, Chiron has offered evidence of its own good faith in its submission accompanying Steimer's declaration. In that submission Chiron tries to clarify the meaning of Steimer's statement regarding expectations of the effectiveness of recombinant immunoassays in 1984. *See* Chiron's Sept. 1990 Supp.Response at 4. Indeed, in that supple-

mental response, Chiron was quite candid regarding its contention, stating that "[a]pplicants are not arguing that some minimal degree of antigenicity was unpredictable. Rather, it is applicants' position that the performance of recombinant env-based immunoassays ... is unexpectedly high in that it was as good as or better than nonrecombinant assays." *Id.* Given the heavy burden that Abbott bears, the court concludes that faced with the evidence of Chiron's good faith and only the minimal evidence of Dr. Steimer's intent to deceive, no reasonable factfinder could conclude by clear and convincing evidence that Steimer had the requisite intent to deceive the PTO in her declaration based on paragraph 4. Accordingly, Abbott cannot meet its burden by relying on this paragraph to support its inequitable conduct defense, and Chiron is entitled to summary judgment on that issue.

C. *Summary*

For the reasons set forth above, the court concludes that there is sufficient evidence in the record for a reasonable factfinder to conclude that the PTO considered the Steimer Declaration in making its final allowability determination. Thus, Abbott is entitled to proceed to trial upon an inequitable conduct defense on some of the alleged misrepresentations/omissions. Specifically, Abbott is entitled to proceed upon, but not itself entitled to summary judgment on, its inequitable conduct defense based upon (1) Dr. Steimer's failure to reference the HBV project; and (2) Dr. Steimer's failure to reference the FeLV project. Abbott cannot, as a matter of law, base its inequitable conduct defense on (1) Steimer's representations as to the status of the HAV project, or (2) Steimer's statement in paragraph 4.

II. *Prior Invention*

A. *Analytical Framework*

[11, 12] In order to prove prior invention, Abbott must establish by clear and convincing evidence that the invention claimed in the '949 patent was made first by another party who had not abandoned, suppressed, or concealed it. 35 U.S.C. § 102(g). "Priority goes to the first party to reduce an invention to practice unless the other party can show that it was first to conceive the invention and that it exercised reasonable diligence in later reducing that invention to practice." *Price v. Symsek*, 988 F.2d 1187, 1190 (Fed.Cir.1993).[18] The testimony of an alleged prior inventor or inventors, standing alone, is insufficient to prove prior invention; it must be corroborated by other evidence. *Id.* at 1194–95. The alleged inventor's laboratory notebooks suffice for purposes of corroboration, even where they are not contemporaneously witnessed by the inventor. *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1378–79 (Fed.Cir.1986), *cert. denied*, 480 U.S. 947, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987).

B. *Analysis*

Chiron filed its original patent application on October 31, 1984, which therefore constitutes the date upon which Chiron constructively reduced the invention to practice.[19] Accordingly, in order to prevail on its prior invention defense, Abbott must establish by clear and convincing evidence that prior to October 31, 1984, someone other than Chiron was the first to conceive of the invention and diligently reduced it to practice.

Abbott contends that both NIH/Centocor and DuPont beat Chiron's invention date.[20] Specifically, Abbott argues that (1) both NIH/Centocor and DuPont conceived of the invention prior to Chiron, (2) NIH/Centocor actually reduced the invention to practice prior to Chiron, and (3) both NIH/Centocor and DuPont diligently reduced the invention to practice in a timely manner.

In response, Chiron contends that there is no evidence of prior invention. Specifically,

18. This rule is sometimes referred to as the "first to conceive, last to reduce to practice" rule.

19. Of course, Chiron is entitled to show that it actually conceived of and reduced the invention to practice prior to that date in order to defeat alleged prior inventors.

20. Abbott also alludes to other possible prior inventors, including, apparently, Genentech. However, Abbott has failed to submit any evidence as to any other putative prior inventors.

Chiron argues that (1) it was the first to conceive of the invention and reduce it to practice, (2) under the doctrine of simultaneous conception and reduction to practice, it prevails because it was the first to reduce the invention to practice, and (3) the alleged prior inventors did not exercise diligence in reducing the invention to practice, and/or abandoned it.

The court must first determine as a matter of law what constitutes conception and reduction to practice in the instant context.

1. *Conception*

[13, 14] Conception is the formulation of a "definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in practice." *Burroughs Wellcome Co. v. Barr Labs., Inc.* 40 F.3d 1223, 1228 (Fed.Cir.1994) (citation omitted). It "necessarily turns on the inventor's ability to describe his invention with particularity," and the idea must be sufficiently formed so that "only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation." *Id.* However, in the ordinary context, an inventor need not know or demonstrate that the invention actually works for conception to be complete—that discovery is part of reduction to practice, not conception. *Id.*

[15] Chiron half-heartedly argues first that conception of the instant invention requires merely having the *idea* for a recombinant immunoassay based on env region polypeptides, and disclosure of that idea to others.[21] In the alternative, Chiron argues that because the instant invention involves isolation of proteins at the genetic level, the doctrine of simultaneous conception and reduction to practice applies, such that conception was not complete until there was actual reduction to practice.[22]

Abbott contends that more is required for conception than just the idea of expressing an immunoreactive polypeptide from the env region of HIV; rather, Abbott argues, a specific DNA fragment that can be used to express an immunoreactive polypeptide must be identified by restriction sites (i.e., a restriction map of the DNA must be created). Abbott also argues that conception in this instance does not require actual gene sequencing of the env region—that the doctrine of simultaneous conception and reduction to practice does not apply, and that all that is required for conception is creation of a restriction map identifying specific DNA fragments that possibly could be immunoreactive.

The court must determine whether conception of the instant invention requires (1) simply formulating the idea of a recombinant immunoassay based on env region polypeptides, as Chiron initially contends, (2) creating a restriction map and identifying specific possibly useful DNA fragments from the env region, as Abbott argues, or, (3) actual reduction to practice, including sequencing and analysis of the env region to ensure that the identified fragments actually are from the env, as Chiron argues in the alternative.

The Federal Circuit has held that

> [a] gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. [citation omitted]. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, *e.g.* encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property.

Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1206 (Fed.Cir.), *cert. denied sub. nom.*, 502 U.S. 856, 112 S.Ct. 169,

21. At oral argument and in supplemental briefing, Chiron essentially abandoned this contention altogether.

22. Chiron further argues that creation of a restriction map was unnecessary for purposes of conception, because it is just a predictable, mechanical step that anyone of ordinary skill could perform.

116 L.Ed.2d 132 (1991). Based on this rationale, the court held that "when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated." *Id.* On the facts of *Amgen*, the court concluded that a putative inventor did not have conception of an invention for cloning a gene, because he had only the general idea for the cloning but had not isolated and did not know the chemical structure (i.e. the nucleotide sequence) of the gene. *Id.* at 1207; *see also Fiers v. Revel*, 984 F.2d 1164, 1169 (Fed.Cir.1993) (holding that it is not sufficient for conception simply to describe a DNA-based invention by its "hoped-for function.").

With respect to chemical compounds, the Federal Circuit has held that conception "includes knowledge of both the specific chemical structure of the compound and an operative method of making it." *Burroughs Wellcome*, 40 F.3d at 1229. In every case in which it analyzed conception of an invention involving DNA encoding a human protein, the Federal Circuit has held that an inventor does not have knowledge of the specific chemical structure (and thus conception) until the inventor knows the nucleotide sequence of the relevant DNA and has a viable method for obtaining it. *Id.*; *Fiers*, 984 F.2d at 1168–69; *Amgen*, 927 F.2d at 1206; *see also Colbert v. Lofdahl*, 21 U.S.P.Q.2d 1068, 1071 (Bd.Pat.App. & Interf.1991). These cases have established what has become known as the doctrine of simultaneous conception and reduction to practice: until experimentation reveals the chemical structure of the protein (i.e. its nucleotide sequence) there is no "conception." Hereinafter, the court will refer to this merging of conception and reduction to practice as "hybrid conception."[23]

The instant case falls squarely within the rationale and holdings of the *Amgen/Burroughs Wellcome* line of cases. The mere idea of a recombinant immunoassay based on env region polypeptides, without knowledge and analysis of the nucleotide sequence of specific env DNA fragments, is, as the court in *Amgen* put it, "simply a wish to know the identity of any material" that would be immunoreactive with HIV, not a "definite and permanent idea of the complete and operative invention." *Amgen*, 927 F.2d at 1206–07; *see also Fiers*, 984 F.2d at 1169 ("conception of a DNA ... requires a definition of that substance other than by its functional utility."). Until the inventor possesses knowledge of both the nucleotide sequence of an HIV fragment from the env, and has an operative method of isolating that fragment, the mere idea that immunoreactive polypeptides from the env region would be capable of serving as an immunoassay (its functional utility) is inadequate as a matter of law to constitute conception.[24]

Abbott's attempt to distinguish the *Amgen/Burroughs Wellcome* line of cases fails. Abbott argues that in *Fiers* and *Amgen*, the invention involved a DNA sequence encoding a *complete* human protein, such that knowledge of the entire sequence from beginning to end was required to ensure that the whole protein was encoded. By contrast, Abbott argues, the instant invention involves only a DNA *fragment*, which Abbott contends can be described adequately by restriction sites alone, without any sequencing knowledge. Based upon a review of all the submissions before the court, including those submitted in response to the tentative order, this court finds this argument unpersuasive. As an initial matter, the Board of Patent Appeals and Interferences has applied the *Amgen/Burroughs Wellcome* doctrine to an invention involving a protein fragment, finding that in order to have the chemical structure of a protein or its fragment, the inventor

23. The court notes that while hybrid conception of the instant invention is equivalent to *actual* reduction to practice, it would constitute only a first step toward *constructive* reduction to practice (via a patent application), which of course has additional requirements such as enablement and best mode. *See* 35 U.S.C. § 112; *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1560 (Fed.Cir. 1991).

24. It is undisputed that it is sequencing, not the creation of restriction maps, that enables scientists to create a *complete* map of the exact order of the nucleotides comprising the DNA and to accurately locate sections of the genome such as the env.

must isolate the DNA molecule and know its nucleotide sequence. *See Colbert*, 21 U.S.P.Q.2d at 1071. Furthermore, in the context of the instant invention, all of the evidence in the record reveals that until the inventor had precisely located the env via sequencing, the inventor could not be certain that any expressed protein was indeed from the env region. Putney Depo. at 34–35; Dina Depo. at 494; Luciw Depo. at 440–41; Chang Depo. at 1088; Weiss Decl. ¶ 15; Wong-Staal Depo. at 182–83. Thus, as in *Amgen* and *Fiers*, where the inventor could not be sure that the complete desired protein was encoded without knowing the entire nucleotide sequence, here the inventor could not be sure that the expressed protein was the desired protein (i.e. from the env) without locating the env region by sequencing.[25]

Under the doctrine of simultaneous conception and reduction to practice, therefore, conception of the instant invention did not occur until there was hybrid conception. Accordingly, in order to prevail on its prior invention defense, Abbott must establish by clear and convincing evidence that a party other than Chiron was first to complete hybrid conception of the invention described in the '949 patent.

2. *Hybrid Conception/Reduction to Practice*

An inventor can demonstrate reduction to practice by establishing either constructive or actual reduction to practice.

[16, 17] The act of filing a patent application constitutes a constructive reduction to practice of the invention described therein. *Hazeltine Corp. v. United States*, 820 F.2d 1190, 1196 (Fed.Cir.1987). However, to constitute constructive reduction to practice, the application must, *inter alia*, satisfy the disclosure requirements of 35 U.S.C. § 112: it must set forth a written description of the invention, enable one of ordinary skill in the art to make and use the invention, and set forth a "best mode" for carrying out the invention. *See Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1560 (Fed.Cir.1991). It is not disputed that Chiron completed the first step in constructively reducing the invention to practice by no later than October 31, 1984, when it filed its patent application.[26]

"Actual reduction to practice requires a showing that the embodiment relied upon as evidence of priority actually worked for its intended purpose." *DSL Dynamic Sciences v. Union Switch & Signal*, 928 F.2d 1122, 1125 (Fed.Cir.1991) (citation omitted). Both parties agree, and the court concludes, that actual reduction to practice (and therefore hybrid conception) of the instant invention occurred when the inventor (1) expressed recombinant proteins (2) known to be from the env region of HIV that (3) were immunoreactive with HIV-infected sera. As part of hybrid conception, the inventor necessarily

25. In its tentative order, this court held that possession of a restriction map was a prerequisite to conception, and knowledge of the nucleotide sequence was not. The court is now persuaded that that conclusion was erroneous. As set forth above, the *Amgen/Burroughs Wellcome* line of cases clearly establish that in the context of genetic compounds, sequencing is a prerequisite to conception, and conception is not complete until there is actual reduction to practice. That holding comports with the evidence in the instant record. There is undisputed testimony in the record that while it is true that a protein can be expressed by one of ordinary skill in the art without having the benefit of any sequencing information once a DNA fragment is defined by its restriction sites, *see* Ghrayeb Depo. at 433–38; Putney Depo. at 34, 50–51; Dina Depo. at 494; 500–01; Luciw Depo. at 439–40, it also is true that without the sequencing information, the inventor cannot be sure that the fragment is from the desired region of the protein, Putney Depo. at 34–35; Dina Depo. at 494; Luciw Depo. at 440–41; Chang Depo. at 1088; Weiss Decl. ¶ 15; Wong-Staal Depo. at 182–83, and thus, under *Amgen*, cannot have conception. Simply possessing a restriction map and expressing a protein on that basis does not tell the inventor that he has expressed the protein he desires to express—only sequencing can provide that crucial information.

26. As this court has held above, under the hybrid-conception doctrine, Chiron completed one aspect of constructive reduction to practice by filing a patent application. There remain further elements of the doctrine of constructive reduction to practice. These additional elements are the subject of a second round of scheduled motions in which Abbott argues, *inter alia*, that the '949 patent is invalid because it fails to enable one of ordinary skill in the art to practice the invention. However, Abbott has not disputed that by the date it filed its patent application, Chiron had achieved hybrid conception of the instant invention.

must have sequenced the env region of HIV and have knowledge of the precise location of the env on the genome to ensure that the expressed proteins were in fact from the env.

3. *Discussion*

[18] Having determined as a matter of law that for purposes of the instant invention, priority goes to the party that first completed hybrid conception, and having established the requirements for hybrid conception, the court must determine on the voluminous factual record whether Abbott necessarily has or could establish by clear and convincing evidence that someone other than Chiron was first to reduce the invention to practice. Exhaustive review of the evidence and briefing submitted by both parties in conjunction with this motion reveals that the technology at issue is highly complex, and the import of actions taken by the various research teams in their race to invent an immunoassay difficult to decipher. Analyzing all of the evidence in the record in the context of the above rulings, the court concludes that neither party is entitled to prevail on summary judgment on Abbott's priority defense.

Abbott can prevail on its prior invention defense only by submitting clear and convincing evidence that some other party completed hybrid conception, either by constructively or actually reducing it to practice, before Chiron did. It is undisputed for purposes of this motion that Chiron completed the first step toward constructively reducing the invention to practice on October 31, 1984, and Chiron argues that it actually reduced the invention to practice in late September 1984.

Abbott does not directly challenge Chiron's alleged October 31, 1984 constructive reduction to practice.[27] Instead, Abbott argues that both NIH/Centocor and DuPont conceived of the invention (under the traditional standard) prior to October 31, 1984 and diligently reduced it to practice thereafter, and that NIH/Centocor actually reduced the invention to practice prior to Chiron. Abbott also argues that NIH/Centocor constructively reduced the invention to practice on October 10, 1984 by filing the '339 application, and thereby reduction to practice prior to Chiron.

In response, Chiron contends that no other party succeeded in actually reducing the invention to practice prior to October 31, 1984, the date of its constructive reduction to practice. Chiron also argues that NIH/Centocor's filing of the '339 application on October 10, 1984 cannot constitute constructive reduction to practice because that application was legally insufficient in several critical ways.

a. *Chiron's September 1984 Work*

Chiron contends that in addition to constructively reducing the invention to practice on October 31, 1984, it actually reduced the invention to practice on September 26, 1984, when Dr. Luciw, the inventor of the '949 patent, claims to have first successfully expressed an HIV polypeptide from the EcoRI-Kpn fragment which tests confirmed was both from the env and immunoreactive with human AIDS sera.

Abbott contends that Chiron's late September 1984 work cannot constitute actual reduction to practice because Chiron lost Luciw's laboratory notebook. Abbott argues that Luciw's testimony is thus the uncorroborated testimony of a putative inventor, which cannot as a matter of law suffice for purposes of priority.

In support of its contention that Dr. Luciw successfully reduced the invention to practice in late September 1984, Chiron submits the following evidence: (1) Luciw's own testimony that on September 26, 1984 he expressed the EcoRI-Kpn fragment of HIV clone pS7c/7D, which he and Dr. Dina confirmed by an immunofluorescence test was a recombinant env protein that was immunoreactive with human AIDS sera; Luciw Depo. at 116-20; Luciw Decl. ¶ 4; (2) Dr. Dina's testimony recalling his and Luciw's successful immunofluorescence test in late September 1984;

27. Abbott is entitled to argue—and does so in the next round of scheduled motions—that the patent ultimately issued on the October 31, 1984 application was not enabling, which would mean that it cannot constitute a constructive reduction to practice. Because the issue of whether the October 31 application is enabling is not properly before the court on this motion, the court cannot resolve it.

Dina Depo. at 165–66; (3) Dr. Sanchez-Pescador's testimony that as part of Luciw's team, he sequenced the entire env region of the HIV genome and knew its boundaries by late September 1984; Sanchez-Pescador Depo. at 39–40; (4) photographs of the tubes Luciw used in his experiment, which bear the labels "SV7c" or "7D", "Eco+Kpn", and which are dated 9/22/94; Luciw Decl. ¶ 4 & Exs. A–C; (5) an excerpt from the notebook of Dr. Barr, who Luciw asked on 9/27/84 to modify the SV7c/7D vector as part of an experiment that was derivative of the expression of the EcoRI-Kpn fragment, as well as a photograph of the tube used for that experiment, which is dated 9/28/84 and bears the label "SV7c/7D env gel; × Eco+Sac"; Luciw Decl. ¶ 6 & Exs. D & E; (6) an excerpt from the notebook of Maryann Wormstead, a Chiron lab tech, which confirms Luciw's statement that he repeated the experiment in early October; Luciw Decl. ¶ 7 & Ex. F; and (7) slides and photographs of the results of the early October repeat experiments; Luciw Decl. ¶ 8 & Exs. G–J. Other than pointing to the absence of Luciw's notebook, Abbott offers no evidence of its own to contradict Luciw's testimony.

Review of the evidence submitted by Chiron reveals that Luciw's testimony is not, as Abbott contends, entirely without corroboration. Certainly, there is substantial circumstantial evidence to corroborate his claim that in late September 1984 he successfully expressed an immunoreactive polypeptide from the env region of HIV. However, the court is deeply troubled by the absence of the one piece of evidence that could directly corroborate (or, perhaps, contradict) Luciw's testimony—his laboratory notebook. Its absence arouses suspicion. Despite the other potentially corroborating evidence in the record, absent the notebook the court cannot and will not determine as a matter of law that Dr. Luciw's late September 1984 work constituted successful reduction to practice. However, considering all of the evidence submitted by Chiron, a reasonable jury certainly *could* reach that conclusion. Therefore, while Chiron is not entitled to summary judgment that it reduced the invention to practice in late September 1984, Abbott also is not entitled to summary judgment on priority unless it can submit clear and convincing evidence that someone else reduced the invention to practice prior to late September 1984, because a reasonable jury could conclude that Chiron reduced to practice on that date.

b. *NIH/Centocor Actual Reduction*

Abbott contends that NIH/Centocor actually reduced the invention to practice prior to October 31, 1984.[28] Specifically, Abbott argues that NIH/Centocor sequenced a segment of HIV's DNA encoding "most" of the env region by late August 1984, that it sequenced the entire env by late August or early September 1984, and that it began successfully expressing immunoreactive polypeptides from the env soon thereafter, as early as mid to late October 1984.

In support of Abbott's contention, there is the following evidence in the record: (1) Dr. Chang met with Dr. Putney on July 26, 1984 to discuss a DNA fragment on the BH-8 restriction map called SstI-HindIII, which they hypothesized to encode the env; Joint Statement ¶ 30; Putney Depo. at 36–41, 48; (2) Chang and Putney discussed using this fragment and subfragments thereof to produce recombinant polypeptides, and over the next few days they prepared written time schedules to memorialize their intentions; Putney Depo. at 36–38, 40–49, 124–26; Abbott Ex. 2E ("Putney Notebook") at 16744–48; Chang Depo. at 875–76; (3) in July 1984 Dr. Ghrayeb had a working hypothesis that the SstI-HindIII fragment contained immunoreactive env polypeptides, and a July 31, 1984 entry in his notebook is entitled "Sequencing of SstI-HindIII fragment," which was a BH-8 fragment that contains approximately 76% of the env region of BH-8; Ghrayeb Depo. at 407–10; Joint Statement ¶¶ 31–32; (4) notebooks kept by Centocor scientists reflect experimentation with the SstI-HindIII fragment beginning in July 1984 and continuing thereafter; Abbott Ex.

28. Because the court has held that conception of the instant invention requires actual reduction to practice, Abbott's argument regarding NIH/Centocor's prior conception under the traditional standard and diligent reduction to practice is irrelevant and will not be addressed.

3, Huang Decl. ¶ 7–10; Abbott Ex. 8A ("Huang Notebook") at 23–220; Abbott Ex. 2G ("Ghrayeb Notebook") at 343–88, 387–91, 425, 437–47; (5) by August 10, 1984, Centocor scientists expressed a polypeptide from the SstI–HindIII fragment that they suspected was an env gene fragment; Putney Depo. at 102–04; Putney Notebook at 16755; (6) by late August 1984 Centocor sequenced all of the BH–8 clone; Chang Depo. at 879; (7) by early September, NIH sequenced all of the BH–8 clone; Ratner Depo. at 18, 29, 112–13; (8) on September 7, 1984, Chang wrote a memorandum about her meeting with Dr. Gallo and Dr. Wong–Staal in which she reported Centocor's "data on the cloning and expression of HTLV–III *env* gene in *E. coli* host cell and its immunoreactivity to AIDS patient serum"; Abbott Ex. 2F; (9) by October 10, 1984, Centocor identified six clones (out of 1000 tested) that "express[ed] HTLV–III–*env*–B–galactosidase fusion proteins (antigens) that cross react with the HTLV–III specific antibody", and two of these clones (4 and 107) were subfragments of the SstI–HindIII fragment; Abbott Ex. 2N ("'339 application") at A4356–57, 4384; Ghrayeb Depo. at 418–24; Ghrayeb Notebook at 441; (10) on October 20, 1984 Chang and Putney discussed the immunoreactivity of clone 121, which is from a subfragment of the SstI–HindIII fragment that encodes a portion of the env region of HIV; Putney Depo. at 120–26; Putney Notebook at 16796–800; (11) on November 29, 1984, Dr. Chang, Dr. Wong–Staal, and others submitted an article to the journal *Nature* which was published on January 24, 1985 as *Complete Nucleotide Sequence of the AIDS Virus, HTLV–III*, 313 Nature 277 (1985); Abbott Ex. 2K; (12) by December 4, 1984 NIH/Centocor determined the immunoreactivity of polypeptides from subfragments of the SstI–HindIII fragment; Putney Notebook at 16837–40; Abbott Ex. 2H; (13) on December 21, 1984, Dr. Chang and others submitted an article to the journal *Science* called *Expression in Escherichia of Open Reading Frame Gene Segments of HTLV–III*, which was published in April 1985, and which states that polypeptides produced from HIV clones (numbers 127, 121 and 113) were immunoreactive, and that these clones were taken from "fragments of HTLV–III DNA derived from BH–10"; Abbott Ex. 2H.

In response, Chiron argues that the NIH/Centocor scientists did not successfully reduce the invention to practice prior to October 31, 1984. Specifically, Chiron argues that prior to October 31, 1984 the NIH/Centocor scientists did not know for sure that the proteins they were expressing were from the env region because they had not successfully sequenced the env and did not know either its precise boundaries or exact location, and that there is no evidence in the record that NIH/Centocor successfully determined immunoreactivity prior to October 31, 1984.

In support of Chiron's argument, there is the following evidence in the record: (1) in August and September 1984, NIH/Centocor was not focussed on just the SstI–HindIII fragment as Abbott argues—they were testing many fragments based on random cloning, not directed expression, because they did not know yet the precise location of the env; Wong–Staal Depo. at 114–15, 132; Chang Depo. at 577–79; Ghrayeb Depo. at 100–01; (2) Dr. Huang's September 3, 1984 positive test for immunoreactivity with the EcorI–HindIII fragment was a false positive—he had no success at immunoreactivity until some unknown date after September 30, 1984; Huang Chiron Decl. ¶¶ 7–10; Huang Supp.Chiron Decl. ¶¶ 4–5; (3) NIH/Centocor's hypothesis as to the precise location of the env was wrong; Huang Chiron Decl. ¶ 13; Shearman Decl. ¶¶ 6–7; Weiss Decl. ¶¶ 5–7; (4) Chang's assertion regarding Centocor's August 1984 sequencing of the env is uncorroborated and directly contradicted in the record by other Centocor scientists, which indicates that Centocor was not in possession of the sequencing information even by late September 1984; Ghrayeb Depo. 80–82, 90–91, 259; Huang Chiron Decl. ¶¶ 11–12; Shearman Decl. ¶ 12; (5) even once NIH/Centocor scientists completed sequencing the env, they still did not know its precise location or boundaries; Ratner Depo. at 158–59; Livak Depo. at 93, 102–03, 148–49; (6) the '339 application itself, filed on October 10, 1984, does not contain accurate sequencing of or correct boundaries for the

env region; '339 Application at Figure 8; Chang Depo. at 278–281; (7) the October 10, 1984 identification of two immunoreactive fragments in the '339 application is not corroborated by any notebook evidence other than one undated note in the Ghrayeb notebook which was not written by Ghrayeb himself, and the immunoblot upon which the claim is based is essentially uninterpretable; Ghrayeb Depo. at 271, 348–49; Weiss Decl ¶¶ 13–15; Chang Depo. at 481–88; (8) Putney's notes from his October 20, 1984 discussion with Chang reflect that the clone they discussed—number 121—was not one of the two clones upon which the '339 application was based and on which Abbott claims NIH/Centocor was focussed; Putney Notebook at A16796.

Careful review of this competing evidence reveals that it is impossible for this court to determine as a matter of law whether NIH/Centocor successfully reduced the invention to practice prior to Chiron. The conflicting testimony and evidence of the actions taken by NIH/Centocor scientists, including whether they actually had sequenced the env in late August 1984, whether as a result of that sequencing they knew its precise location, and whether they had determined immunoreactivity prior to October 31, as well as the competing interpretations of the import of those actions, constitute the classic genuine dispute of material fact which must be resolved by a jury presented with all of the evidence and with an ability to assess witness credibility. Furthermore, as explained above, there is conflicting evidence in the record as to the precise date Chiron actually reduced the invention to practice.

Accordingly, neither Abbott nor Chiron is entitled to prevail on summary judgment on Abbott's priority defense based on the work performed by NIH/Centocor.

c. *The '339 Application*

Abbott contends that by filing the '339 application on October 10, 1984, NIH/Centocor constructively reduced the invention to practice on that date. The court notes at the outset that while the October 10, 1984 date would not entitle Abbott to summary judgment because it does not beat Chiron's possible late September 1984 date, the court must nonetheless address the merits of this argument, as a jury could reject the September 1984 date and conclude that Chiron did not reduce to practice until its constructive reduction to practice on October 31, 1984.

Pursuant to 35 U.S.C. § 112, for a patent application to constitute a constructive reduction to practice, it must enable one of ordinary skill in the art to make and use the invention, meet the written description test, and set forth the best mode of invention. 35 U.S.C. § 112; *see also Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1560 (Fed.Cir.1991). "That some [further] experimentation is necessary does not constitute a lack of enablement; the amount of experimentation, however, must not be unduly extensive." *Amgen*, 927 F.2d at 1212 (citing *Atlas Powder Co. v. E.I. duPont De Nemours & Co.*, 750 F.2d 1569, 1576 (Fed.Cir.1984)).

Chiron argues that the '339 application cannot constitute constructive reduction to practice under section 112 because it fails to meet the enablement requirement, fails to provide an adequate written description of the invention, and fails to set forth a best mode.

i. *Enablement*

[19] In the instant context, in order to meet the enablement requirement, the '339 application must disclose to one of ordinary skill in the art how to make and use recombinant HIV proteins from the env that are immunoreactive with human AIDS serum. Chiron argues that the '339 application fails to meet the enablement requirement because each of its three embodiments (1) requires starting materials that are neither described in the application nor available in the prior art; (2) relies on information about the structure and nucleotide sequence of HIV that was neither disclosed in the application nor available in the prior art; and (3) contains additional fatal flaws.

(a) *Starting Material*

The '339 application sets forth three methods of making recombinant HIV env proteins for diagnostic use. *See* '339 Application at A4353–59. Of the three embodiments, the first two use as starting material an HIV

"clone," and the third uses as starting material "fragments of [HIV] DNA of approximately 200–500 b[ase]p[airs]." *Id.*

Chiron argues that the application fails to describe the source or names of, or the process for obtaining, these clones and fragments. Chiron further argues that as of October 10, 1984, there was no method of cloning HIV available in the art, and that Dr. Chang failed to deposit the unnamed clones mentioned in the application with the ATCC. Thus, Chiron concludes that one of ordinary skill in the art attempting to practice the '339 application would be defeated at the outset by an in ability to obtain or produce the necessary starting material. In response, Abbott relies primarily on the undisputed fact that Dr. Gallo and Dr. Wong-Staal had deposited the BH-10 and BH-8 clones with the ATCC by August 22, 1984. In addition, Abbott argues that deposit with the ATCC is unnecessary, and that once HIV had been identified, cloning was merely an application of routine methods already available in the literature. Thus, Abbott concludes that one of ordinary skill in the art attempting to practice the application either could have obtained starting material from the ATCC or simply cloned the starting material based on routine science.

The evidence put forth by both parties with respect to starting material is quite weak. Neither party submits any expert testimony as to the ability of one skilled in the art to practice the invention based on the description of the starting material contained in the '339 application. Instead, both parties ask the court to interpret the application on its face. Ordinarily, in such circumstances the court would find that there is a genuine issue of material fact to be resolved by a jury. However, in the instant context, it is clear to the court that the application fails as a matter of law to set forth sufficient information to enable one of ordinary skill to obtain or make the necessary starting material. First, nowhere in the application are the clones or fragments it references as starting material identified by name. In its papers Abbott refers to the deposit of BH-10 and BH-8 with the ATCC, but neither of those clones is mentioned by name in the application itself; instead, the application uses only generic words like "clone" and "fragment" without more identifying information. Thus, it is undisputed that a scientist would not even know from the application what clones to look for at the ATCC. Further, even if one assumes that cloning was routine in 1984—and there is no expert testimony or other real evidence in the record to this effect [29]—it is undisputed by the parties that different clones of HIV vary slightly from one another. Thus, even if cloning HIV was routine, a scientist capable of doing so would not necessarily be able to practice the invention because any particular clone created might not match the clones and fragments referenced in the '339 application.

Accordingly, the court concludes that based on the evidence in the record, no reasonable jury could conclude that the '339 application was a constructive reduction to practice, because the application fails to set forth sufficient information to enable one of ordinary skill to obtain or make the necessary starting material. While this conclusion alone entitles Chiron to summary judgment that the '339 application does not constitute a constructive reduction to practice as a matter of law, the court will address each of the remaining arguments raised by Chiron regarding that application in the interest of thoroughness.

(b) *Sequence Information*

Chiron argues that the '339 application also fails the enablement requirement because it relies on information about the structure and nucleotide sequence of HIV that it does not disclose and which was not available in the prior art. Specifically, Chiron points out that the invention requires that the expressed proteins come from the env region of HIV, and argues that the application contains no accurate description of the nucleotide se-

29. In support of its argument that cloning of HIV was routine in 1984, Abbott cites a single article from 1982 describing the cloning of the HTLV-1 virus. *See* Abbott Ex. 8K. There is no evidence in the record, however, and Abbott cites none, to suggest that the mere fact that scientists had determined how to clone one retrovirus means that cloning of a different retrovirus therefore was routine.

quence or location of the env on the genome. Chiron argues that Figure 8 of the application, which purports to set forth the "nucleotide sequences for [HIV] DNA which encompasses the *env* region", does not indicate the location of that sequence on the overall genome, nor which portions of it represent the env. Chiron further cites to Dr. Chang's admission that of the 3112 nucleotides set forth in Figure 3, only 883 are from the env, Chang Depo. at 278–80, which itself is actually 2588 nucleotides long, such that Figure 3 sets forth only about one-third of the actual env and fails to distinguish the env nucleotides from non-env nucleotides.[30] Thus, Chiron argues, assuming a scientist could express proteins based on the process described in the application, the application provides that scientist with no basis for determining if the expressed proteins are from the env, which is a critical facet of reducing the invention to practice.

In response, Abbott argues that nucleotide sequences are not necessary for enablement, that the '339 application discloses methods of practicing the invention without sequencing, and that because the one-third of the env for which the application discloses a nucleotide sequence actually contains fragments that are immunoreactive, it does not matter that the application fails to specifically locate the env or set forth its entire sequence. In support of its argument, Abbott offers only the declaration by its putative expert that the portion of the env sequence the application does disclose actually contains three fragments that encode immunoreactive polypeptides. Jack Decl. ¶¶ 7–8.

Once again, although the record is fairly short on evidence for the court to evaluate, Chiron's argument must prevail. This court has held as a matter of law that actual reduction to practice of the instant invention requires the inventor to know that the expressed proteins are from the env, which requires both possession of the nucleotide sequence and identification of the precise location the env. It is undisputed that without such information, a scientist cannot be certain that the expressed proteins are from the env. It also is readily apparent (and essentially undisputed) that obtaining the sequence information would in fact require extensive experimentation—indeed, obtaining accurate sequencing of HIV was a major (and complex) feature of all of the various immunoassay projects.

One of ordinary skill in the art attempting to practice the invention described in the '339 application would have no way of knowing for sure whether the expressed proteins were from the env, because the application does not set forth the complete sequence of the env or locate its precise boundaries. While Dr. Jack's testimony that a scientist might actually express a protein from the env using the process set forth in the application is correct, that possibility is irrelevant to enablement because the scientist could not be reasonably certain that it was in fact from the env without extensive further experimentation. Indeed, a scientist who expressed a protein would be misled by Figure 3 into believing it was from the env even if it was not, as that figure purports to set forth the env sequence when in fact most of the nucleotides on it are *not* from the env.

Accordingly, the court concludes that based on the evidence in the record, no reasonable jury could conclude that the '339 application was a constructive reduction to practice, because the application fails either to set forth a complete nucleotide sequence of the env or to locate its precise boundaries.

(c) *Additional Alleged Flaws*

Chiron argues that each of the three embodiments also has additional flaws that preclude the '339 application from constituting constructive reduction to practice.

As to the first embodiment, Chiron argues that none of the fragments identified for use in that embodiment were actually from the env region, such that a scientist practicing that embodiment would not express an env protein. As support for this argument, Chiron cites Dr. Chang's testimony in which she

30. Chiron also points out that the first publication of the sequence information for the NIH clones was in the January 24, 1985 article entitled *Complete Nucleotide Sequence of the AIDS Virus, HTLV-III*, 313 Nature 277 (1985), which was not submitted for publication until November 29, 1984 by Dr. Chang and others at NIH/Centocor.

admits that following the first embodiment literally, a scientist would get "nothing," and that either a crucial sentence was missing from the '339 application or one of the sentences had to be deleted for the first embodiment to work. Chang Depo. at 543–45.

In response, Abbott argues that in fact the first embodiment does enable one of ordinary skill to express immunoreactive polypeptides from the env region. In support of this argument, Abbott cites the declaration testimony of a putative expert, who states that one of ordinary skill practicing the first embodiment would in fact express proteins from the env region of HIV. Jack Decl. ¶¶ 5–8, 10.

Because the parties submit conflicting evidence as to whether one of ordinary skill in the art practicing the first embodiment could express an immunoreactive env polypeptide, there is a classic dispute of material fact which cannot be resolved by the court on summary judgment.

As to the second embodiment, Chiron points out that the random cloning method the '339 employs is based on three DNA fragments, identified in the application as "spanning the *env* gene." '339 application at A4355. Chiron cites Dr. Chang's concession that of these three fragments, one contains no env, one contains almost no env, and one does not contain all of the env. Chang Depo. at 572–77. Chiron also argues that while a scientist practicing this embodiment could actually express immunoreactive polypeptides from the env, the embodiment fails to provide that scientist a means for distinguishing between env and non-env proteins. Chang Depo. at 590–91; Ghrayeb Depo. at 221–22, 268–69; Weiss Decl. ¶ 11. Chiron finally argues that the second embodiment is not saved by the suggestion that following actual expression of a protein, the scientist must sequence the fragment to ascertain whether it is from the env, because the '339 application does not itself set forth the sequence of the env. Furthermore, knowing the sequence of the fragment alone is not sufficient to determine whether it is from the env. Weiss Decl. ¶ 15; Chang Depo. at 1088; Wong-Staal Depo. at 182–83. In response to this argument, Abbott has offered no evidence or argument with respect to whether the second embodiment is enabling.

Because Abbott has offered no evidence or argument as to whether the second embodiment is enabling, the only evidence in the record reveals that a scientist practicing the second embodiment has no basis for determining whether the proteins expressed pursuant to that embodiment are from the env. Accordingly, Chiron is entitled to summary judgment that the second embodiment is not enabling.

As to the third embodiment, Chiron argues that it wholly fails to describe what its starting material is, and its use of gene-specific DNA probes to locate env proteins fails because for probes to work, the location of the target env or its sequence must be known, and the application fails to set forth either the location or sequence of the env. Weiss Decl. ¶¶ 8–9; Chang Depo. at 552–56. In response, Abbott once again has offered no evidence or argument with respect to whether the third embodiment is enabling.

Because Abbott has offered no evidence or argument as to whether the third embodiment is enabling, the only evidence in the record reveals that despite the description in the third embodiment of gene-specific DNA probes, a scientist practicing the third embodiment nonetheless has no basis for determining whether the proteins expressed pursuant to that embodiment are from the env. Accordingly, Chiron is entitled to summary judgment that the third embodiment is not enabling.

ii. *Written Description*

In the instant context, in order to meet the written description requirement the '339 application must show that as of the filing date, the putative inventors possessed a means for producing a recombinant clone encoding the env region of HIV that was used to produce an env protein immunoreactive with human AIDS serum. *See In re Wertheim*, 541 F.2d 257, 262 (C.C.P.A.1976).

Chiron argues that the '339 application fails to satisfy the written description requirement because nothing in the application indicates that NIH/Centocor actually had used a recombinant clone to produce an im-

munoreactive env protein. As support for this argument, Chiron cites to the numerous flaws in the application detailed above as evidence that NIH/Centocor could not have successfully practiced the invention themselves at the time of the application. In response, Abbott once again raises no argument and offers no evidence to counter Chiron's contention.

While Abbott has not cited any evidence in response to Chiron's argument, the court nonetheless cannot grant Chiron summary judgment on this issue. The court has held, in section II.B.3.b above, that a jury must determine when NIH/Centocor actually reduced the invention to practice. Accordingly, despite the flaws in the '339 application detailed above, and Abbott's failure to address this argument head on, the court simply cannot say as a matter of law that NIH/Centocor was not in possession of the invention on October 10, 1984 when it filed the '339 application.

iii. *Best Mode*

[20] The best mode rule requires an inventor to set forth in a patent application the best mode known to the applicant for practicing the invention. The purpose of this requirement is to prevent the inventor from obtaining patent protection without actually having to set forth a means for its successful practice. *See Advanced Cardiovascular Sys., Inc. v. Scimed Life Sys., Inc.*, 20 U.S.P.Q.2d 1791, 1793, 783 F.Supp. 413 (D.Minn.1991). An act of concealment violates the best mode rule. *Engel Indus. Inc. v. Lockformer Co.*, 946 F.2d 1528, 1531 (Fed. Cir.1991).

[21] Chiron argues that the '339 application violates the best mode rule because it fails to set forth an enabling description of the necessary starting materials despite the fact that its inventors had possession of that information. In support of this argument, Chiron cites the evidence this court found persuasive above, namely, that the application does not set forth adequate information for obtaining or making the necessary starting material. In addition, Chiron points to evidence in the record that no later than August 1984, Dr. Gallo and Dr. Wong-Staal, two of the putative inventors, knew how to clone HIV, but failed to include this information in the application. *See* Krevans Decl. Ex. 4, Hahn et al., *Molecular Cloning and Characterization of the HTLV-III Virus Associated with AIDS*, 312 Nature 166 (1984); Joint Statement ¶ 60 (Gallo and Wong-Staal filed the '306 application on August 22, 1984, setting forth "the process for molecularly cloning the complete genome of the HTLV-III virus."). Chiron argues that the failure to disclose this information in the application constitutes concealment, and thus violates the best mode rule. In response to this argument, Abbott has once again stood mute.

The only evidence in the record is that NIH/Centocor possessed the means for obtaining the necessary starting material to practice the invention, but failed to disclose that information in the '339 application. The court concludes as a matter of law that this failure constitutes concealment and thus violates the best mode rule. Accordingly, the court holds that no reasonable jury could conclude that the '339 application was a constructive reduction to practice because the application fails to set forth a best mode.[31]

d. *DuPont*

[22] Abbott finally argues that DuPont also conceived of the invention under the traditional standard prior to Chiron and then diligently reduced it to practice on December 28, 1984. Specifically, Abbott argues that DuPont successfully sequenced the entire env region of HIV and located its precise boundaries by September 1, 1984, that it expressed env proteins in late November 1984, and that those proteins proved immunoreactive on December 28, 1984.

Because the court has followed the Federal Circuit in holding that conception of the instant invention did not occur until there was actual reduction to practice, Abbott's contention that DuPont conceived of the invention under the traditional standard prior to Chiron and then diligently reduced it to practice

31. The court notes, without drawing any conclusions, that the '339 application is still pending [illegible] PTO. Nothing in the record explains why no decision has been reached on the application.

misses the mark. In all of the voluminous briefing and argument on this motion, both oral and written, Abbott has never argued (or offered any evidence) that DuPont actually reduced the invention to practice prior to Chiron—it's sole contention with respect to DuPont is that it *diligently* reduced the invention to practice after having traditional conception prior to Chiron. There is no evidence in the record that DuPont satisfied the hybrid-conception requirement prior to Chiron. Therefore, Abbott's argument regarding DuPont's diligent reduction to practice is unavailing. Indeed, Abbott itself repeatedly asserts that it "is undisputed" that DuPont actually reduced the invention to practice in late December 1984, well after October 31, 1984.

While it is true that the precise date of Chiron's reduction to practice (actual or constructive) necessarily remains an open question, the court cannot and will not make arguments or posit theories on behalf of Abbott that it has chosen not to make. Because Abbott has never argued or offered evidence that DuPont actually reduced the invention to practice prior to Chiron, it cannot proceed upon that theory to support its priority defense. Accordingly, Chiron is entitled to summary judgment on Abbott's priority defense based on DuPont's work.

C. *Summary*

For the reasons set forth above, the court concludes that conception of the instant invention occurred only with hybrid conception—conception *plus* actual reduction to practice—and that actual reduction to practice required expression of recombinant proteins known to be from the env region of HIV that were immunoreactive with HIV-infected sera. Neither party is entitled to prevail on summary judgment on Abbott's prior invention defense. Chiron is entitled to proceed upon its contention, but not itself entitled to summary judgment, that it actually reduced the invention to practice as early as late September 1984, and that it constructively reduced the invention to practice on October 31, 1984. Abbott is entitled to proceed upon its argument, but not itself entitled to summary judgment, that NIH/Centocor actually reduced the invention to practice prior to Chiron, but it cannot as a matter of law proceed upon its argument that the '339 application constituted constructive reduction to practice. Chiron is entitled to summary judgment that Abbott cannot proceed upon its theory that DuPont actually reduced the invention to practice prior to Chiron.

CONCLUSION

For the foregoing reasons, IT IS HEREBY ORDERED that:

(1) Abbott's motion for summary judgment on its inequitable conduct defense is DENIED;

(2) Chiron's motion for summary judgment on Abbott's inequitable conduct defense is DENIED in part and GRANTED in part, as set forth above;

(3) Abbott's motion for summary judgment on its prior invention defense is DENIED;

(4) Chiron's motion for summary judgment on Abbott's prior invention defense is DENIED in part and GRANTED in part, as set forth above;

(5) Because of the numerous opportunities the parties have had to argue and brief the issues involved in these motions, the court will entertain no motions to reconsider this order.

IT IS SO ORDERED.

Ravinder Kumar SHARMA, Petitioner,

v.

Janet RENO, United States Attorney General, and Thomas Schiltgen, District Director, Immigration and Naturalization Service, Respondents.

No. C 95-2175 SBA.

United States District Court, N.D. California.

Sept. 29, 1995.

Alien petitioned for habeas corpus relief to review determination of Immigration and

CURRICULUM VITAE

NAME: Flossie Wong-Staal

Citizenship: United States

Education:

1968 - B.A. in Bacteriology, (magna cum laude), University of California, Los Angeles, California

1972 - Ph.D. in Molecular Biology, University of California, Los Angeles, California

Brief Chronology of Employment:

1990 - Present	Florence Riford Chair in AIDS Research, Professor of Medicine & Biology, University of California, San Diego, California
1982 - 1989	Chief, Molecular Genetics of Hematopoietic Cells Section, National Cancer Institute
1978 - 1981	Senior Investigator, National Cancer Institute, Bethesda, Maryland
1976 - 1978	Cancer Expert, National Cancer Institute, Bethesda, Maryland
1975 - 1976	Visiting Associate, National Cancer Institute, Bethesda, Maryland
1973 - 1975	Fogarty Fellow, National Cancer Institute, Bethesda, Maryland
1972 - 1973	Postdoctoral Fellow, University of California, San Diego Medical Center, California
1970 - 1972	Research Assistant, University of California, Los Angeles, California
1969 - 1970	Teaching Assistant, University of California, Los Angeles, California

Societies:

American Society for Virology, Charter Member

Honors and Awards:

1967 - 1968	Regents Scholarship, UCLA
1968	Phi Beta Kappa
1968	Regents Fellowship, UCLA
1972	Women Graduates of the Year Award, UCLA
1985	Visiting Professorship, Institute of General Pathology, First University of Rome, Italy
1987	Outstanding Scientific Award, The Chinese Medical and Health Association
1991	The Excellence 2000 Award, United States Pan Asian American Chamber of Commerce and the Organization of Chinese American Women
1994	Election to membership of Academia, Sinica, Taipei, Taiwan
1994	Election to membership, Institute of Medicine, National Academy of Sciences, Washington, D.C.
1995	Election to honoary membership, American Society of Clinical Investigation

Research Interests:

Molecular biology of human pathogenic viruses, cancer and AIDS; mechanisms of gene regulation; novel approaches to gene therapy; molecular vaccines.

Editorial Boards:

Gene Analysis Techniques, 1984-1993
Cancer Letters, 1984-1994
Leukemia, 1987-present
Cancer Research, 1987
Journal of AIDS Research and Human Retroviruses (Section Editor), 1987-1995
DNA and Cell Biology (Section Editor), 1987-1991
Microbial Pathogenesis, 1987-1990
AIDS: An International Journal, 1987-present
Journal of Acquired Immunodeficiency Syndrome, 1988-present
Oncogene, 1988-present
Virology, 1988-present
Journal of Virology, 1990-present
HIV: Advances in Research and Therapy, 1990 - present
Gene Therapy, 1993-present

Scientific Committees and Advisory Panels:

Association for AIDS Research, Italy
American Institute of Biological Sciences, DOD
Leukemia Society Panel, 1987
HHS Executive AIDS Vaccine Committee 1989
Genetic Variation Advisory Panel for NIAID AIDS Program 1989-1990
International Scientific Committee:
- Second International AIDS Conference, Paris, 1986
- Third International AIDS Conference, Washington D.C., 1987
- Fourth International AIDS Conference, Stockholm, 1988
- Fifth International AIDS Conference, Montreal, 1989
- Sixth International AIDS Conference, San Francisco, 1990
- Seventh International AIDS Conference, Florence, 1991
- Eighth International AIDS Conference, Amsterdam, 1992
- Ninth International AIDS Conference, Berlin, 1993
- Tenth International AIDS Conference, Yokohama, Japan, 1994

Co-chair for Basic Science Track, Tenth International AIDS Conference, Yokohama, Japan
Maryland Institute of Biotechnology, Advisory Panel
Center for AIDS Research, Stanford University, School of Medicine, Advisory Panel
Center for AIDS Research, Harvard AIDS Institute, Advisory Panel
Center for AIDS Research, University of Washington, Advisory Panel
NIAID Board of Scientific Counselors, 1994-present
National Task Force for AIDS Drug Development, 1994-1995
- Co-chair, AIDS Drug Development Subcommittee

Health Science Policy Board, Institute of Medicine, National Academy of Sciences.
- 1994-present.

Science Board, FDA, 1995-present

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Organizers: Takis Papas, Myron Essex, **Flossie Wong-Staal**, Robert Gallo and Steve Kozurides

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Organizers: Samuel Broder and **Flossie Wong-Staal**

Genetic Structure and Regulation of HIV. Harvard AIDS Institute, 1990.
Organizers: William Haseltine and **Flossie Wong-Staal**

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G. Meyers, S.F. Josephs, A.B. Rabson, T.F. Smith and **F. Wong-Staal**, (Eds.) Los Alamos National Laboratory, Los Alamos, New Mexico.

Special Lectures

Gene Therapy for AIDS and Cancer. ***Women in Science Day, M.D. Anderson Cancer Center, 1996***

Molecular Biology of HIV. ***The Robert T. Wong Lectureship. University of Hawaii, 1996***

A Combinatorial Approach to AIDS Gene Therapy. ***The Keynote Lecture Series, Harvard AIDS Institute, 1995.***

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II. Invited Articles, Book Chapters, etc.

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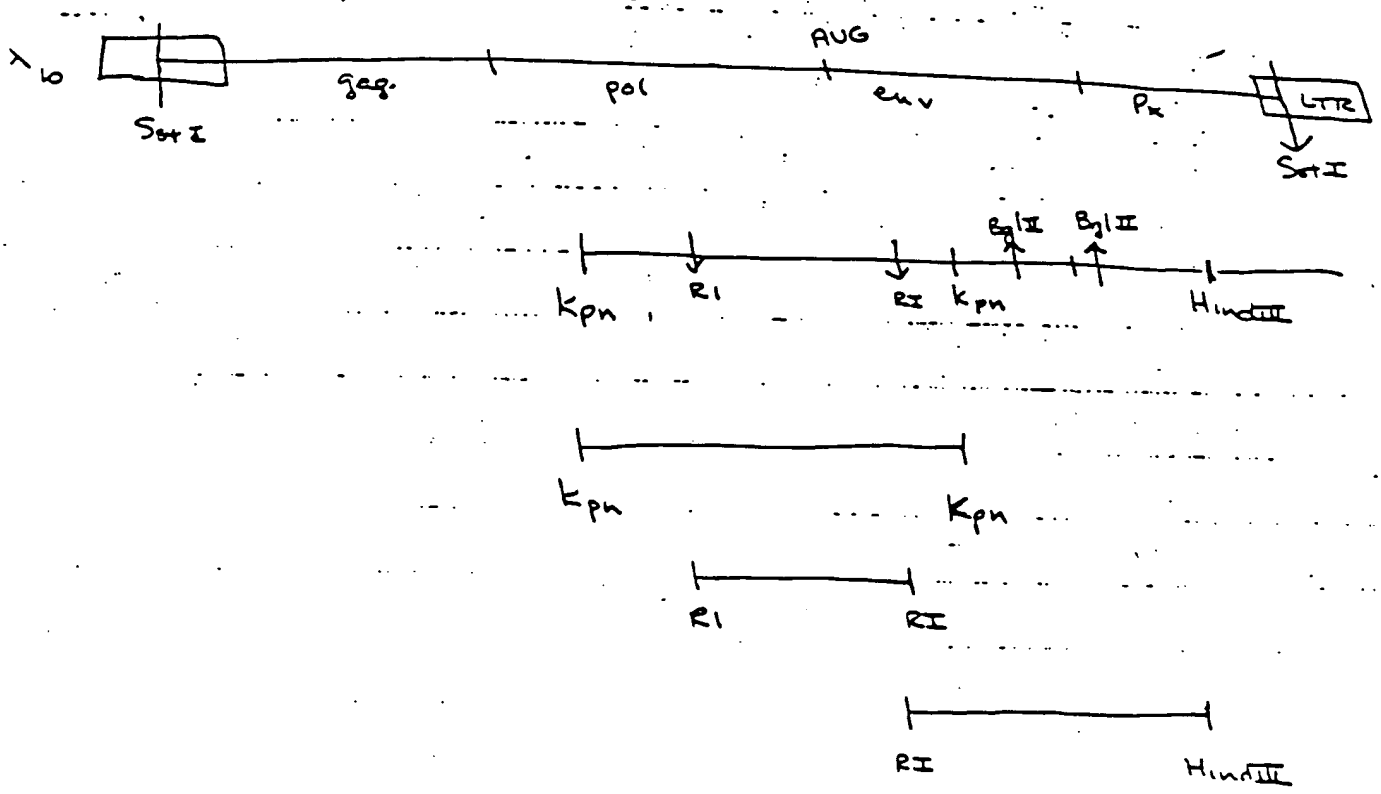
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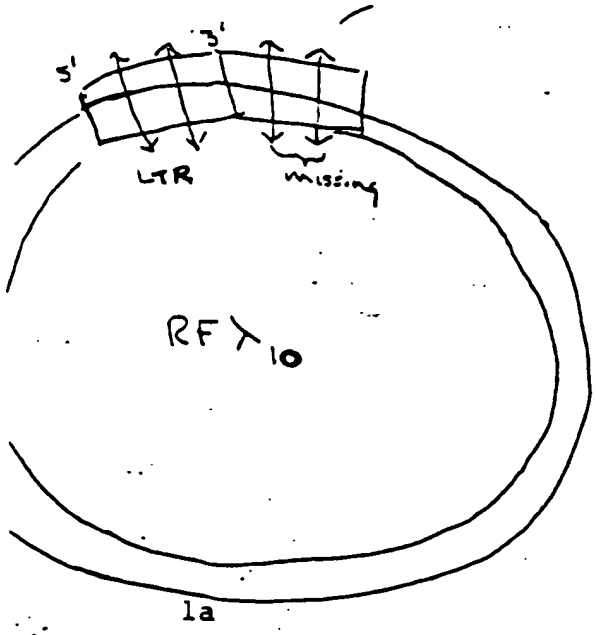
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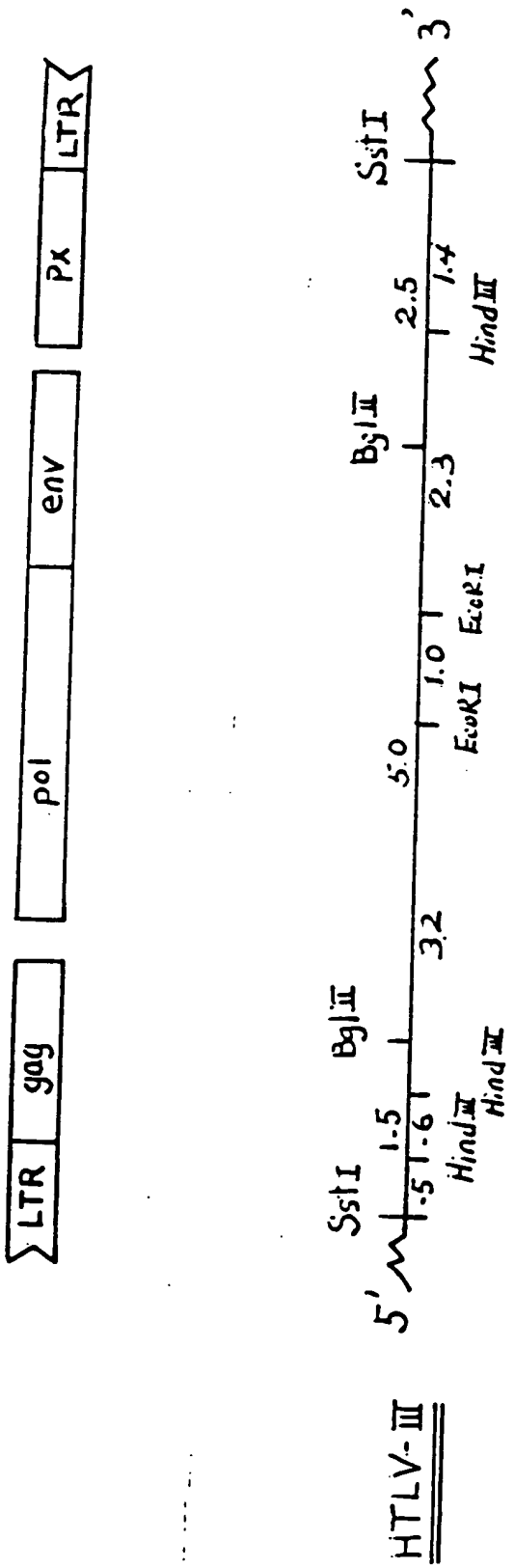
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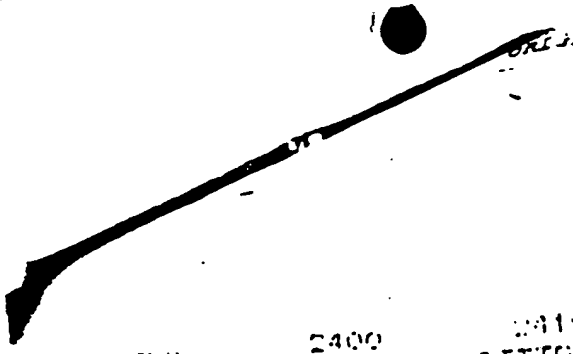

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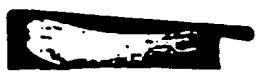

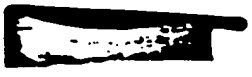

UNITED STATES APPLICATION FOR LETTERS PATENT

Inventor(s): Nancy T. Chang

Attorney's Docket No.: CTR84-7

Title: CLONING AND EXPRESSION OF HTLV-III DNA

EXPRESS MAIL Mailing Label No. B15656704

Date of Deposit October 10, 1984

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American Type Culture Collection
12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)881-2600 Telex: 898-055 ATCCNORTH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

National Institutes of Health, National Cancer Institute
Building 37, Room 6A17
9000 Rockville Pike
Rockville, Maryland 20205
Attention: Dr. Flossie Wong-Staal

Deposited on Behalf of: National Institute of Health, National Cancer Institute

Identification Reference by Depositor:	ATCC Designation
λ EH-10 recombinant phage clone of HTLV-III in λ g & Wes λ B	40125
λ EH-5 recombinant phage clone of HTLV-III in λ g & Wes λ B	40126
λ EH-8 recombinant phage clone of HTLV-III in λ g & Wes λ B	40127

The deposits were accompanied by: ___ a scientific description ___ a proposed taxonomic description indicated above.

The deposits were received July 30, 1984 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

___ We will inform you of requests for the strains for 30 years.
X We will not inform you of requests for the strains.
___ The strains are available to the scientific public upon request as of

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above were tested March 4, 1987. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC: Bobbie A. Brandon
(Mrs.) Bobbie A. Brandon, Head, ATCC Patent Depository

Date: March 6, 1987
cc: James A. Oliff, Esq.

Form BP 4/9

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THE UNITED STATES OF AMERICA

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

October 31, 1995

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APPLICATION NUMBER: *06/659,339*
FILING DATE: *October 10, 1984*
TITLE OF INVENTION:
CLONING AND EXPRESSION OF HTLV-III DNA
INVENTOR(S):
CHARLES F. MCNEELY T.

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

Sandra Allen

SANDRA ALLEN
Certifying Officer

REGULAR UTILIT[redacted]

Form PTO-436
(Rev 8/78)

APPLICATION H. b ..ECOME ABANDONED. THIS NOTICE MAILED:

SERIAL NUMBER (Series of 1979)	PATENT DATE	PATENT NUMBER
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SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
06/659,339	10/10/84	436		~~320~~ 127	

APPLICANTS
NANCY T. CHANG, PACLI, PA.

CONTINUING DATA********
VERIFIED

FOREIGN/PCT APPLICATIONS********
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Foreign priority claimed / 35 USC 119 conditions met / Verified and Acknowledged	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☐ yes ☐ no / ☐ yes ☐ no / Examiner's Initials	→	PA	6	40	22	$ 525.00	CTR84-7

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TITLE
CLONING AND EXPRESSION OF HTLV-III DNA

U.S. DEPT. of COMM.-Pat. & TM Office — PTO-436L (Rev. 10-78)

PARTS OF APPLICATION FILED SEPARATELY	PREPARED FOR ISSUE

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CLONING AND EXPRESSION OF HTLV-III DNA

Description

Technical Fields

This invention is in the fields of biology and virology and in particular relates to human T cell leukemia virus - type III (HTLV-III).

Background Art

The term human T cell leukemia-lymphoma virus (HTLV) refers to a unique family of T cell tropic retroviruses. Such viruses play an important role in the pathogenesis of certain T cell neoplasms. There are presently three known types of HTLVs. One subgroup of the family, HTLV-type I (HTLV-I) is linked to the cause of adult T-cell leukemia-lymphoma (ATLL) that occurs in certain regions of Japan, the Caribbean and Africa. HTLV-type II (HTLV-II) has been isolated from a patient with a T-cell variant of hairy cell leukemia. M. Popovic et al., Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS, Science 224:497-

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HTLV-type III (HTLV-III) has been isolated from many patients with acquired immune deficiency syndrome (AIDS). It refers to prototype virus isolated from AIDS patients. Groups reported to be at greatest risk for AIDS include homosexual or bisexual males; intravenous drug users and Haitian immigrants to the United States. Homosexual or bisexual males having heterosexual contacts,

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hemophiliacs who receive blood products pooled from donors and recipients of multiple blood transfusions are also at risk. Clinical manifestations include severe, unexplained immune deficiency which generally involves a depletion of helper T lymphocytes. These may be accompanied by malignancies and infections. The mortality rate for those with AIDS is high. A less severe form of AIDS also exists, in which there may be lymphadenopathy and depressed helper T cell counts; there is not, however, the devasting illness characteristic of full-blown AIDS. There are many individuals, who are classified as having early AIDS (pre-AIDS), who exhibit these signs. It is not now possible to predict who among them will develop the more serious symptoms.

Much of the evidence implicates HTLV-III as the etiological agent of the infectious AIDS. First, there is consistent epidemiology; greater than 95% of the patients with AIDS have antibodies specific for HTLV-III. Second, there has been reproducible identification and isolation of virus in this disease; more than 100 variants of HTLV-III have been isolated from AIDS patients. Third, there has been transmission of the disease to normal healthy individuals who received blood transfusions from infected blood donors.

HTLV-III has been shown to share several properties with HTLV-I and HTLV-II but also to be morphologically, biologically and antigenically distinguishable. R.C. Gallo et al., Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and At Risk for AIDS. Science, 224:500-503. (1984). For example,

HTLV-III has been shown to be antigenically related to HTLV-I and HTLV-II by demonstrating cross-reactivity with antibodies to HTLV-I and HTLV-II core proteins, P24 and P19, and envelope antigens and by nucleic acid cross-hybridization studies with cloned HTLV-I and HTLV-II DNAs. However, unlike HTLV-I and HTLV-II, it lacked the ability to infect and transform T cells from normal umbilical cord blood and bone marrow in vitro, and has the cytopathic effect on infected cells only.

Like the RNA genome of other retroviruses, the RNA genome of HTLV-III contains three genes which encode viral proteins: 1) the gag gene, which encodes the internal structural (nucleocapsid or core) proteins; 2) the pol gene, which encodes the RNA-directed DNA polymerase (reverse transcriptase); and 3) the env gene, which encodes the envelope glycoproteins of the virion. In addition, the HTLV-III genome contains a region designated Px, located between the env gene and the 3' LTR, which appears to be involved in functional killing of the virus.

At this time, AIDS is still difficult to diagnose before the onset of clinical manifestations and impossible to treat or even prevent.

Summary of the Invention

This invention is based upon applicant's cloning of HTLV-III DNA in recombinant/vector host systems capable of expressing immunoreactive HTLV-III polypeptides. In one embodiment, an immunoreactive protein coded for by an env gene sequence of HTLV-III has been produced by these recombinant

DNA methods. This polypeptide is immunoreactive with sera of patients having acquired immunodeficiency syndrome or antibodies to HTLV-III. The polypeptide expressed has been isolated.

In another embodiment of the invention, immunoreactive polypeptides produced by the recombinant DNA methods are employed in the production of antibodies, including monoclonal antibodies, reactive with the polypeptides. Such antibodies form the basis for immunoassay and diagnostice techniques for detecting HTLV-III, particularly in body fluids such as blood, saliva, urine, etc.

In another embodiment of the invention, DNA probes are formed from DNA sequences coding for portions of the HTLV-III genome. Such DNA probes can also be employed in detecting the presence of HTLV-III in blood or other fluids.

Diagnostic kits including immunoreactive polypeptides, DNA probes, etc. can also be produced to include any of the products of this invention.

Brief Description of the Figures

Figure 1 is a representation of HTLV-III DNA. Figure 1a shows sites at which the genome is cut by the restriction enzyme SstI and Figure 1b shows the fragments of HTLV-III genome produced through the action of restriction enzymes Kpn, EcoRI and Hind III.

Figure 2 is a representation of HTLV-III DNA and the location of restriction enzyme sites in the genome.

Figure 3 shows nucleotide sequences for HTLV-III DNA which encompasses the env region.

Figure 4 is an immunoblot showing the position on an SDS polyacrylamide gel of HTLV-III env-Beta-galactosidase fusion proteins.

Best Mode of Carrying Out the Invention

The envelope glycoprotein is the major antigen recognized by the antiserum of AIDS patients. In this respect, HTLV resembles other retroviruses, for which the envelope glycoprotein is typically the most antigenic viral polypeptide. In addition, the neutralizing antibodies are generally directed toward the envelope glycoprotein of the retrovirus. Serum samples from 88 percent to 100 percent of those with AIDS have been shown to have antibodies reactive with antigens of HTLV-III; the major immune reactivity was directed against p41, the presumed envelope antigen of HTLV-III. Antibodies to core proteins have also been demonstrated in serum of AIDS patients, but are evidently not as effective an indicator of infection as is the presence of antibodies to envelope antigen.

The p41 antigen of HTLV-III has been difficult to characterize because the viral envelope is partially destroyed during the process of virus inactivation and purification. The present invention responds to the great need to characterize the antigenic component of the HTLV-III virus--and thus provide screening, diagnostic and preventive products and methods--in several ways.

First, the present invention relates to the isolation of genes of HTLV-III which encode

immunoreactive polypeptides; identification of the nucleotide sequence of these genes; introduction of DNA sequences specific to these viral DNA sequences into appropriate vectors to produce viral RNA and the formation of DNA probes. These probes are comprised of sequences specific to HTLV-III DNA and are useful, for example, for detecting the same HTLV-III DNA sequences in body fluids (e.g., blood).

Second, the present invention relates to HTLV-III polypeptides which are produced by translation of the recombinant DNA sequences encoding HTLV-III proteins. Polypeptides which are so produced and which are immunoreactive with serum from AIDS patients are referred to as recombinant DNA-produced immunoreactive HTLV-III polypeptides. They include, but are not limited to, antigenic HTLV-III core and envelope polypeptides which are produced by translation of the recombinant DNA sequences specific to the gag and the env DNA sequences encoding HTLV-III core proteins and envelope glycoproteins, respectively. They also include the polypeptides which are produced by translation of the recombinant DNA sequences specific to the Px genes of HTLV-III. The polypeptides may be used as vaccines for the prevention of AIDS. The methods of producing the polypeptides are also a subject of this invention, as are diagnostic methods based on these polypeptides.

Third, the present invention also relates to antibodies against the immunoreactive HTLV-III polypeptides which are the subject of this invention. These antibodies are the basis for assays

relating to the diagnosis of AIDS or the presence of HTLV-III in body fluids.

In one embodiment of this invention, genetic engineering methods are used to isolate DNA sequences of HTLV-III which encode immunoreactive HTLV-III polypeptides, such as the core protein and the envelope glycoprotein, and to identify the nucleotides which comprise those sequences. The proviral genes integrated into host cell DNA are molecularly cloned and the nucleotide sequences of the cloned provirus is determined.

An E. coli expression library of HTLV-III DNA is constructed; in this library are vectors harboring HTLV-III DNA sequences. The HTLV-III genome is cloned and cuts are then made in the cloned HTLV-III genome with restriction enzymes to produce DNA fragments. (Figures 1 and 2) HTLV-III DNA fragments of approximately 200-500bp are isolated from agarose gel, end repaired with T_4 polymerase and ligated to linker DNA. The linker ligated DNA is then treated with a restriction enzyme, purified from agarose gel and cloned in an expression vector. Examples of the expression vectors used are: OmpA, pIN (A,B and C), lambda pL, T7, lac, Trp, ORF and lambda gt11. In addition, mammalian cell vectors such as pSV28pt, pSV2neo, pSVdhfr and VPV vectors, and yeast vectors, such as GALI and GAL10, may be used.

The bacterial vectors contain the lac coding sequences, into which HTLV-III DNA can be inserted for the generation of B-galactosidase fusion protein. The hybrid molecules are then introduced into bacteria (e.g., E.coli); those cells which take up a

vector containing HTLV-III DNA are said to be transformed. The bacteria are plated on top of MacConkey agar plates in order to verify the phenotype of clone. If functional B-galactosidase is being produced, the colony will appear red.

Bacterial colonies are also screened with HTLV-III DNA probes containing the DNA regions of interest (e.g., HTLV-III gag and env DNA sequences). This results in identification of those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

This identification of cells harboring the HTLV-III DNA sequences makes it possible to produce HTLV-III polypeptides which are immunoreactive with HTLV-III specific antibody. The cells from the selected colonies are grown in culture under conditions conducive to allowing the expression of the hybrid protein. The culture is spun down and the resulting cell pellet broken. The total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 2) Western blot analyses are also carried out on the clones which screened positive. Such analyses are carried out using serum from AIDS patients, with the result that it is possible to identify those clones expressing HTLV-III env-B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody.

In another embodiment of this invention, lambda $_{10}$ clones harboring HTLV-III DNA are cloned

from the replicated form of the virus. As the retrovirus is replicating, double stranded DNA is being produced. Cuts are made in the cloned HTLV-III DNA with the restriction enzyme SstI. (Figure 1a) Because there are two SstI recognition sites within the LTR of HTLV-III DNA, one LTR region is not present in the cloned DNA sequence removed from the $lambda_{10}$ vector. As a result, a small (approximately 200 bp) fragment of the HTLV-III DNA is missing.

The resulting DNA is linearized and fragments are produced by digesting the linearized genomic DNA spanning the env gene region with restriction enzymes. For example, fragments are produced using Kpn or EcoRI plus HindIII, as shown in Figure 1b. The resulting 2.3kb KpnI-KpnI fragments; 1.0kbEcoRI-EcoRI fragments and 2.4Kb EcoRI-HindIII fragments are isolated by gel electrophoresis and electro-elution. These fragments are randomly sheared to produce fragments. The fragments thus produced are purified from agarose gel and DNA fragments between about 200-500 bp are eluted.

The eluted 200-500bp DNA fragments are end filled through the use of E. coli T_4 polymerase and blunt end ligated into an open reading frame expression (ORF) vector, such as pMR100. This ligation may occur at the SmaI site of the pMR100 vector, which contains two promoter regions, hybrid coding sequences of lambdaCI gene and lacI-LacZ gene fusion sequence. In the vector, these are out of frame sequences; as a result, the vector is nonproductive. The HTLV-III DNA is inserted into the vector; the correct DNA fragments will correct the reading

frame, with the result that CI-HTLV-III-B-galactosidase fusion proteins are produced. The expression of the hybrid is under the control of the lac promoter.

Based on the sequence of pMR100, it appears that if a DNA fragment insert cloned into the SmaI site is to generate a proper open reading frame between the lambdaCI gene fragment and the lac-7 fragment, the inserted DNA must not contain any stop codons in the reading frame set by the frame of the lambdaCI gene.

The hybrid molecules are then introduced into E. coli. The bacteria are plated on MacConkey agar plates to verify the phenotype of the clone. If functional B-galactosidase is being produced, the colony will appear red. The colonies are also screened with HTLV-III DNA probes, for the purpose of identifying those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

The cells from the selected colonies are grown in culture. The culture is spun down and the cell pellet broken. Total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 4)

Western blot analyses are also carried out on the clones which screened positive. Sera from AIDS patients are used, thus making it possible to identify those clones which express the HTLV-III-env-B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody.

1000 clones were screened by this method; 6 were positive.

Because of the nature of the pMR100 cloning vehicle, a productive DNA insert should also be expressed as a part of a larger fusion polypeptide. HTLV-III env gene containing recombinant clones was identified by colony hybridization. The production of larger fusion polypeptides bearing functional B-galactosidase activity was verified by phenotype identification on MacConkey agar plates; by B-galactosidase enzyamatic assays and by analysis on 7.5% SDS-polyacrylamide gels. Immunoreactivity of the larger protein with antibody to HTLV-III was assessed by western blot analysis using serum from AIDS patients. These large fusion proteins also reacted with anti-B-galactosidase and anti-CI antiserum. This finding is consistent with the hypothesis that they are proteins of CI-HTLV-III-lacIZ.

The open reading frame insert fragment of HTLV-III is further analyzed by DNA sequencing analysis. Because one of the two BamHI sites flanking the SmaI cloning site in pMR100 is destroyed in the cloning step, positive clones are digested with restriction enzymes HindIII and claI to liberate the inserted HTLV-III DNA fragment. The HTLV-III ORF inserts are isolated from the fusion recombinant and cloned into M13 sequencing cloning vector mp18 and mp19 digested with HindIII and AccI. DNA sequences of the positive ORF clones are then determined.

In another embodiment of this invention, fragments of HTLV-III DNA of approximately 200-500

bps are isolated from agarose gel, end repaired with T_4 polymerase and ligated to EcoRI linker. The EcoRI linker ligated DNA is then treated with EcoRI purified from 1% agarose gel and cloned in an expression vector, gt11. This vector contains lac Z gene coding sequences into which the foreign DNA can be inserted for the generation of B-galactosidase fusion protein. The expression of the hybrid gene is under the control of lac repressor. The lac repressor gene, lac I, is carried on a separate plasmid pMC9 in the host cell, E. coli Y1090. AIDS patient serum was used to probe the gt11 library of HTLV-III genome DNA containing 1.5×10^4 recombinant phage. In a screen of 5000 recombinants, 100 independent clones that produced strong signals were isolated. The positive recombinant DNA clones were further characterized for their specific gene expression. Rabbit hyperimmune serum against P24 was also used to identify the gag gene specific clones. Nick-translated DNA probes of specific HTLV-III gene, specifically the gag gene, env gene and Px gene were used to group the positive immunoreactive clones into specific gene region.

Recombinant clones that produced strong signals with AIDS serum and contain insert DNA spanning the HTLV-III env gene region were examined in detail by mapping their insert with restriction enzymes and DNA sequencing analysis.

Another embodiment of this invention relates to the formation of RNA and RNA probes specific to the HTLV-III DNA of this invention. DNA sequences which are an entire gene or segment of a gene from HTLV-III are inserted into a vector, such as a T7 vector.

In this embodiment, the vector has the Tceu promoter from the T cell gene 10 promoter and eleven amino acids from the T cell gene 10 protein.

The vectors are then used to transform cells, such as E. coli. The T7 vector makes use of the T7 polymerase, which catalyzes RNA formation and recognizes only T7 promoter, which is the site where RNA polymerase binds for the initiation of transcription. This vector does not, however, recognize E. coli promoter. As a result, if HTLV-III DNA sequences are inserted after the promoter and polymerase genes of the T7 vector, which recognizes them to the exclusion of other signals, and a terminator is placed immediately after the HTLV-III DNA sequences, the T7 vector will direct manufacture RNA complementary to the HTLV-III DNA insert.

Monoclonal antibodies reactive with HTLV-III envelope polypeptide are produced by antibody-producing cell lines. The antibody-producing cell lines may be hybridoma cell lines commonly known as hybridomas. The hybrid cellsa re formed from the fusion of cells which produce antibody to HTLV-III envelope polypeptide and an immortalizing cell line, that is, a cell line which imparts long term tissue culture stability on the hybrid cell. In the formation of the hybrid cell lines, the first fusion partner - the antibody-producing cell - may be a spleen cell of an animal immunized against HTLV-III envelope polypeptide. Alternatively, the antibody-producing cell may be an anti-HTLV-III envelope polypeptide lymphocyte obtained from the spleen, peripheral blood, lymph nodes or other tissue. The

second fusion partner - the immortal cell - may be a lymphoblastoid cell or a plasmacytoma cell such as a myeloma cell, itself an antibody-producing cell but also malignant.

Murine hybridomas which produce monoclonal antibodies against HTLV-III envelope polypeptide are formed by the fusion of mouse myeloma cells and spleen cells from mice immunized against the polypeptide. To immunize the mice, a variety of different immunization protocols may be followed. For instance mice may receive primary and boosting immunizations of the purified polypeptide. The fusions are accomplished by standard procedures. Kohler and Milstein, (1975) Nature (London) 256, 495-497; Kennet, R., (1980) in Monoclonal Antibodies (Kennet et al., Eds. pp. 365-367, Plenum Press, NY).

The hybridomas are then screened for production of antibody reactive with envelope polypeptide.

Another way of forming the antibody-producing cell line is by transformation of antibody-producing cells. For example, a B lymphocyte obtained from an animal immunized against HTLV-III envelope polypeptide may be infected and transformeed with a virus such as the Epstein-Barr virus in the case of human B lymphocytes to give an immortal antibody-producing cell. See, e.g., Kozbor and Rodor (1983) Immunology Today 4(3), 72-79. Alternatively, the B lymphocyte may be transformed by a transforming gene or transforming gene product.

The monoclonal antibodies against HTLV-III envelope polypeptide are produced in large quantities by injecting antibody-producing hybridomas into the peritoneal cavity of mice and, after an

appropriate time, harvesting the ascites fluid which contains very high titer of homogenous antibody and isolating the monoclonal antibodies therefrom. Xenogeneic hybridomas should be injected into irradiated or athymic nude mice. Alternatively, the antibodies may be produced by culturing cells which produce HTLV-III envelope polypeptide in vitro and isolating secreted monoclonal antibodies from the cell culture medium.

This invention will now be further illustrated by the following examples. They are not intended to be limiting in any way.

EXAMPLE 1
PREPARATION OF SONICATED DNA FRAGMENTS

10 ug of gel purified HTLV-III restriction fragments were sonicated to fragment size on average of 500 bps. After sonication, the DNA was passed through a DEAE-cellulose column in 0.1XTBE in order to reduce the volume. The DEAE-bound DNA was washed with 5 ml of 0.2 M NaCl-TE (2 M NaCl, 10 mm Tris HCl pH 7.5, 1 mM EDTA) and then eluted with 1 M NaCl-TE, and ethanol precipitated. The size range of the sonicated DNA was then determined on 1.2% agarose gel. DNA fragments of desired length (200-500 bps) was eluted from the gel. T4 DNA polymerase was used to fill in and/or trim the single strand DNA termini generated by the sonication procedure. DNA fragments were incubated with T4 polymerase in the absence of added nucleotides for five minutes at 37°C to remove nucleotides from 3' end and then all 4 nucleotide precursors were added to a final

concentration of 100 uM and the reaction mixture was incubated another 30 minutes to repair the 5'-end single stranded overhang. The reaction was stopped by heat inactivation of the enzyme at 68°C for 10 minutes. DNA was phenol extracted once, ethanol precipitated and resuspended in TE.

EXAMPLE 2

CLONING OF RANDOM SHEARED DNA FRAGMENTS

The sonicated blunt end repaired HTLV-III DNA fragments were ligated into the SmaI site of the ORF expression vector pMR100 and transformed into host cell LG90 using standard transformation procedures. B-galactosidase positive phenotype of the transformant were identified by plating the transformed cell on ampicillin (25 ug/ml) containing McConkey agar plates and scoring the phenotype after 20 hours at 37°C.

EXAMPLE 3

HYBRID PROTEIN ANALYSIS

Ten milliliter samples of cells from an overnight saturated culture grown in L broth containing ampicillin (25 ug/ml) were centrifuged, the cell pellet was resuspended in 500 ul of 1.2 fold concentrated Laemmli sample buffer. The cells were resuspended by vortexing and boiling for 3 minutes at 100°C. The lysate was then repeated by being forced through a 22 guage needle to reduce the lysate viscosity. Approximately 10 ul of the protein samples were electrophoresed in 7.5% SDS-PAGE (SDS-polyacrylamide) gels.

Electrophoretic transfer of proteins from SDS-PAGE gels to nitrocellulose paper was carried out according to Towbin et. al.. After the transfer, the filter was incubated at 37°C for two hours in a solution of 5% (w/v) nonfat milk in PBS containing 0.1% antifoam A and 0.0001% merthiolate to saturate all available protein binding sites. Reactions with AIDS antisera were carried out in the same milk buffer containing 1% AIDS patient antisera that had been preabsorbed with E. coli lysate. Reactions were performed in a sealed plastic bag at 4°C for 18-24 hours on a rotatory shaker. Following this incubation, the filter was washed three times for 20 minutes each at room temperature in a solution containing 0.5% deoxycholic, 0.1 M NaCl, 0.5% triton X-100, 10 mm phosphate buffer pH 7.5 and 0.1 mM PMSF.

To visualize antigen-antibody interactions, the nitrocellulose was then incubated with the second goat antihuman antibody that had been iodinated with ^{125}I. The reaction with the iodinated antibody was carried out at room temperature for 30 minutes in the same milk buffer as was used for the first antibody. The nitrocellulose was then washed as previously described and exposed at -70°C using Kodak XAR5 film with an intensifying screen.

EXAMPLE 4

SCREENING OF THE HTLV-III ORF LIBRARY BY COLONY HYBRIDIZATION

E. coli LG90 transformants were screened with HTLV-III DNA probes containing the DNA regions of interest (e.g. HTLV-III gag, env or Px gene specific

sequences). Colonies were grown on nitrocellulose filter and screened according to the procedure of Grunstein and Hogness by using a nick-translated HTLV-III DNA as hybridization probe.

The DNA fragment was in general exercise by restriction endonuclease digestion, gel purified, and ^{32}P-labeled to a specific activity of 0.5×10^8 cpm/ug by nick-translation (Rigby, P.W.J. et al., J. Mol. Biol. 113, 237 (1977). Duplicate nitrocellulose filters with DNA fixed to them were prehybridized with 6xSSC (0.9 M NaCl/0.09 M sodium citrate, pH 7.0), 5X Denhardt's solution (Denhardt's solution: 0.02% each of polyvinylpyrrolidone, Ficoll and bovine serum albumin) 10 ug of denatured sonicated E. coli DNA per ml at 55°C for 3-5 hours. The filters were then placed in a fresh sample of the same solution to which the denatured hybridization probe had been added. Hybridization was permitted to take place at 68°C for 16 hours. The filters were washed repeatedly in 0.3XSSC at 55°C, and then exposed to x-ray film.

Industrial Applicability

This invention has industrial applicability in screening for the presence of HTLV-III DNA in body fluids and the diagnosis of AIDS.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substantces and procedures described herein. Such equivalents are considered to be within the

scope of this invention and are covered by the following claims.

CLAIMS

1. Immunoreactive HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III cDNA.

2. A polypeptide of Claim 1 wherein said HTLV-III cDNA encodes an *env* gene sequence.

3. A polypeptide of Claim 2 wherein which is immunoreactive with sera of patients with acquired immunodeficiency syndrome.

4. Isolated HTLV-III envelope polypeptide.

5. Isolated cDNA encoding an HTLV-III gene.

6. cDNA of Claim 5 encoding the HTLV-III *env* gene.

7. Isolated cDNA encoding for an HTLV-III polypeptide which is immunoreactive.

8. Isolated cDNA of Claim 7 coding for an envelope polypeptide which is immunoreactive.

9. A DNA probe comprising a DNA sequence coding a portion of the HTLV-III genome.

10. A DNA probe of Claim 9 wherein the DNA sequence encodes at least a portion of the *env* gene.

11. A hybrid protein comprising an HTLV-III polypeptide linked to at least one other polypeptide.

12. A hybrid protein of Claim 11 comprising an HTLV-III polypeptide linked to an indicator polypeptide.

13. A hybrid protein of Claim 12 wherein said indicator polypeptide comprises beta-galactosidase.

14. An isolated RNA transcript of the env gene of HTLV-III.

15. An isolated RNA transcript of Claim 14 having a label which emits a detectable signal.

16. An isolated RNA transcript of Claim 15 wherein said label comprises a radioisotope.

17. A recombinant vector containing HTLV-III DNA capable of expression upon insertion into host cells.

18. [redacted]pA vector containing HTLV-III cDNA.

19. p[redacted]100 vector containing HTLV-III cDNA.

20. A method of producing HTLV-III polypeptide, comprising the steps of:

a. cleaving HTLV-III cDNA to produce DNA fragments;

b. inserting the DNA fragments into an expression vector to form a recombinant vector;

c. transforming an appropriate host cell with the recombinant vector; and

d. culturing the transformed host cell under conditions sufficient for expression of the polypeptide coded for by the inserted HTLV-III DNA.

21. A method of Claim 20 wherein the cleaving step comprises digesting the HTLV-III cDNA with restriction endonucleases to produce restriction fragments of cDNA.

22. A method of Claim 20 wherein the cleaving step comprises shearing the HTLV-III cDNA to produce cDNA fragments.

23. A method of producing HTLV-III envelope polypeptide, comprising the steps of:

a. cleaving HTLV-III genomic cDNA with the restriction endonuclease SstI;

b. digesting the cleaved cDNA with restriction endonucleases sufficient to generate restriction fragments which encompass at least a portion of the env gene;

c. isolating the restriction fragments;

d. producing DNA fragments of about 200-500 base pairs in length from the restriction fragments;

e. isolating the DNA fragments of about 200-500 base pairs;

f. inserting the isolated fragments into the open reading frame expression vector pMR100 for production of hybrid proteins comprising an env gene product and beta-galactosidase;

g. transforming lac z⁻ E. coli cells with the vector;

h. plating the transformed cells on MacConkey agar plates, maintaining the plates under conditions sufficient for the formation of colonies and selecting cell colonies exhibiting a red color;

i. culturing transformed cells from the selected colonies under conditions which allow expression of the hybrid protein;

j. obtaining cellular protein from the cultured transformed cells;

k. separating the cellular protein obtained;

l. contacting the separated protein with sera from AIDS patients to identify protein which is immunoreactive with the sera; and

m. isolating the immunoreactive protein.

24. A method of Claim 23, further comprising the step of separating the env gene expression product from the remainder of the hybrid protein.

25. A fusion protein produced by the method of Claim 23.

26. A HTLV-III envelope polypeptide produced by the method of Claim 24.

27. Antibody specifically reactive with HTLV-III envelope polypeptide.

28. An antibody of Claim 27 which is monoclonal.

29. Anibody specifically reactive with HTLV-III polypeptide produced by recombinant DNA techniques.

30. An antibody of Claim 29 which is monoclonal.

31. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III polypeptide produced by recombinant DNA techniques.

32. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III envelope polypeptide.

33. An immunoassay of Claim 32 wherein said antibody is monoclonal.

34. A method for detecting the presence of HTLV-III in a bodily fluid comprising the steps of:

a. contacting an immunoadsorbent comprising a solid phase having an antibody which specifically binds HTLV-III polypeptide with the bodily fluid;

b. separating the immunoadsorbent and the fluid;

c. contacting the immunoadsorbent with a labeled antibody which specifically binds HTLV-III polypeptide; and

d. measuring the amount of label associated with the immunoadsorbent to determine the presence of HTLV-III.

35. An assay kit comprising an antibody which reacts specifically iwth HTLV-III polypeptide bound to a solid phase and a labeled antibody which reacts specifically HTLV-III polypeptide.

36. A method of determining the presence of antibodies against HTLV-III in a bodily fluid comprising the steps of:

a. contacting an immunoadsorbent comprising an HTLV-III polypeptide bound to a solid phase with a bodily fluid;

b. separating the immunoadsorbent from the bodily fluid;

c. contacting the immunoadsorbent with a labeled HTLV-III polypeptide; and

d. determining the amount of labeled polypeptide bound to immunoadsorbent as an indication of antibody to HTLV-III.

37. A kit for determining the presence of antibody against HTLV-III in a bodily fluid comprising:

a. an immunoadsorbent comprising a HTLV-III polypeptide bound to a solid phase; and

b. labeled HLTV-III polypeptide.

38. A method of detecting HTLV-III nucleic acid in a bodily fluid comprising the steps of:

a. adsorbing the nucleic acid in a bodily fluid onto an adsorbent;

b. denaturing the adsorbed nucleic acid;

c. contacting the adsorbed nucleic acid with a HTLV-III DNA or RNA probe; and

d. determining if the probe hybridizes with the adsorbed nucleic acid.

39. A method of Claim 38 wherein the bodily fluid is a cell lysate.

40. A hybridoma cell line which produces antibody specifically reactive with HTLV-III envelope polypeptide.

CLONING AND EXPRESSION OF HTLV-III DNA

Abstract

The production of immunoreactive polypeptides from HTLV-III by recombinant DNA methods is disclosed. Such polypeptides can be employed in immunoassays to detect HTLV-III.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Declaration for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

CLONING AND EXPRESSION OF HTLV-III DNA

the specification of which (check one)

/ / is attached hereto.

/XX/ was filed on October 10, 1984 as Application Serial No. 659,339 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

			Priority Claimed	
(Number)	(Country)	(Day/Month/Year filed)	/ / Yes	/ / No
(Number)	(Country)	(Day/Month/Year filed)	/ / Yes	/ / No
(Number)	(Country)	(Day/Month/Year filed)	/ / Yes	/ / No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing date) (Status, patented, pending, abandoned)

(Application Serial No.) (Filing date) (Status, patented, pending, abandoned)

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

I also hereby grant additional Powers of Attorney to the following attorney(s) and/or agent(s) to file and prosecute an international application under the Patent Cooperation Treaty based upon the above-identified application, including a power to meet all designated office requirements for designated states.

David E. Brook Registration No. 22,592
James M. Smith Registration No. 28,043
Leo R. Reynolds Registration No. 20,884

all of Hamilton, Brook, Smith and Reynolds, Two Militia Drive, Lexington, Massachusetts 02173;

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617-861-6240

Attorney's
Docket No.: CTR84-7

Patent No. Nancy T. Chang
Filed or Issued: 659,339
For: October 10, 1984
CLONING AND EXPRESSION OF HTLV-III DNA

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(c) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:

[X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Centocor, Inc.

ADDRESS OF CONCERN 244 Great Valley Parkway
Malvern, PA 19355

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled CLONING AND EXPRESSION OF HTLV-III DNA by inventor(s) Nancy T. Chang

described in

[ ] the specification filed herewith

[X] application serial no. 659,339, filed October 10, 1984.

[ ] patent no. _____, issued _____.

exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____

ADDRESS _____

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATIC

NAME _____

ADDRESS _____

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATIC

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Vincent R. Zurawski, Jr.

TITLE OF PERSON OTHER THAN OWNER Executive Vice President and Technical Direct

ADDRESS OF PERSON SIGNING 244 Great Valley Parkway

Malvern PA 19355

SIGNATURE _____ DATE November 6, 1984

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature Nancy T. Chang

Date NOV 6. 1984

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Full name of second joint inventor, if any

Second Inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of third joint inventor, if any

Third Inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of fourth joint inventor, if any

Fourth Inventor's signature

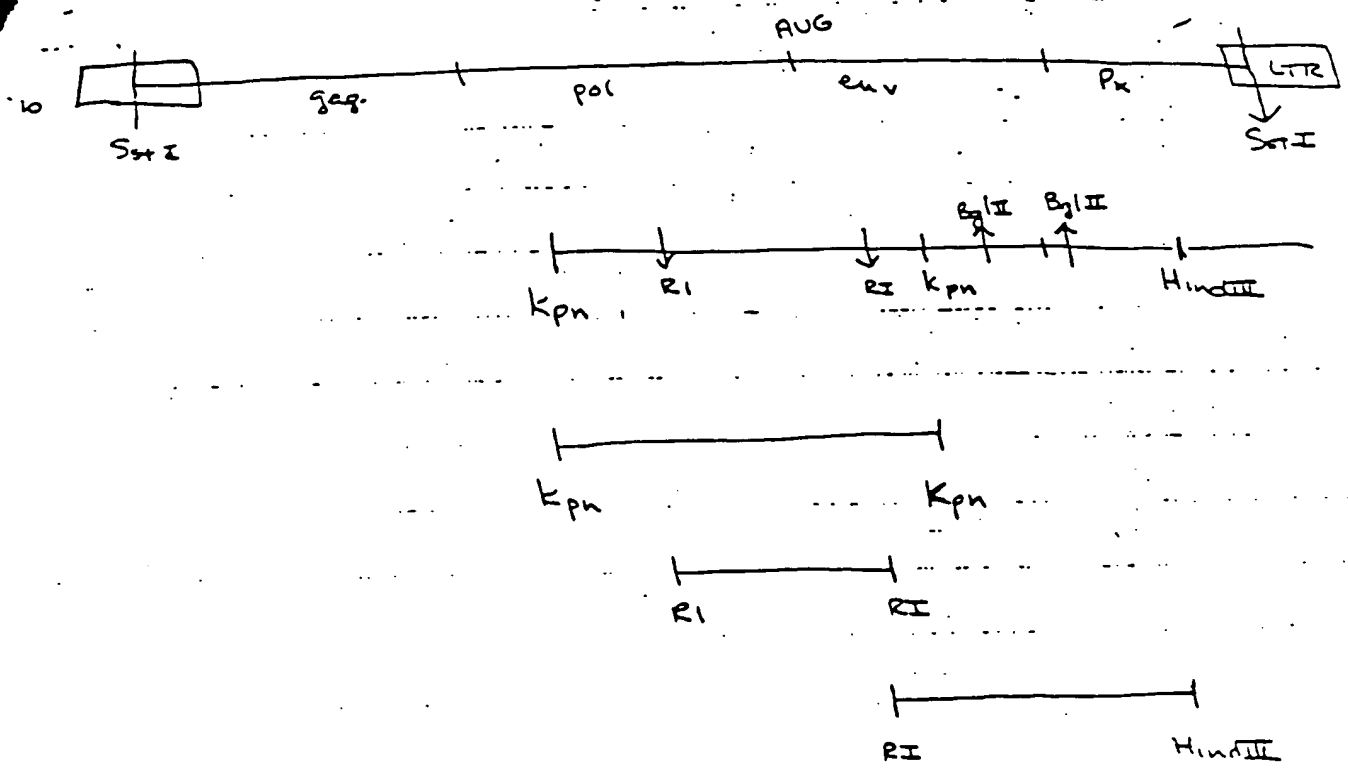
Date

Residence

Citizenship

Post Office Address

E59339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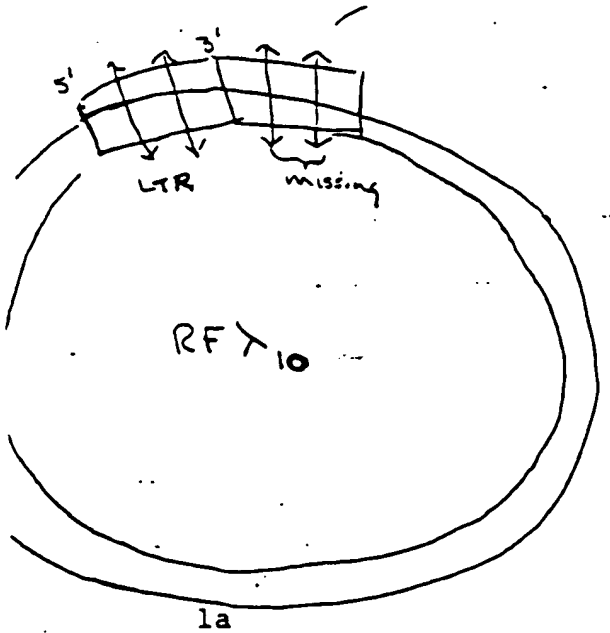
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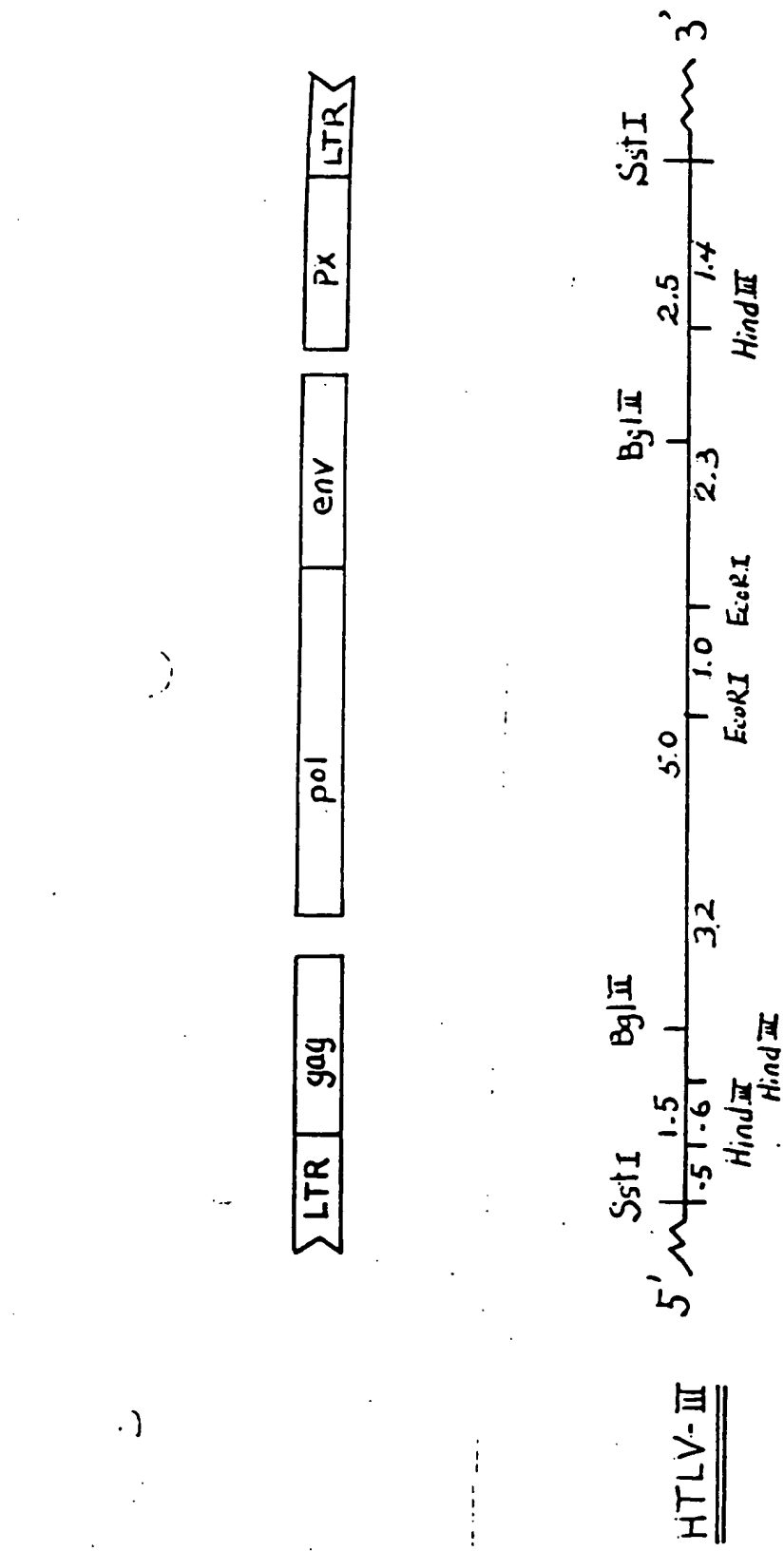
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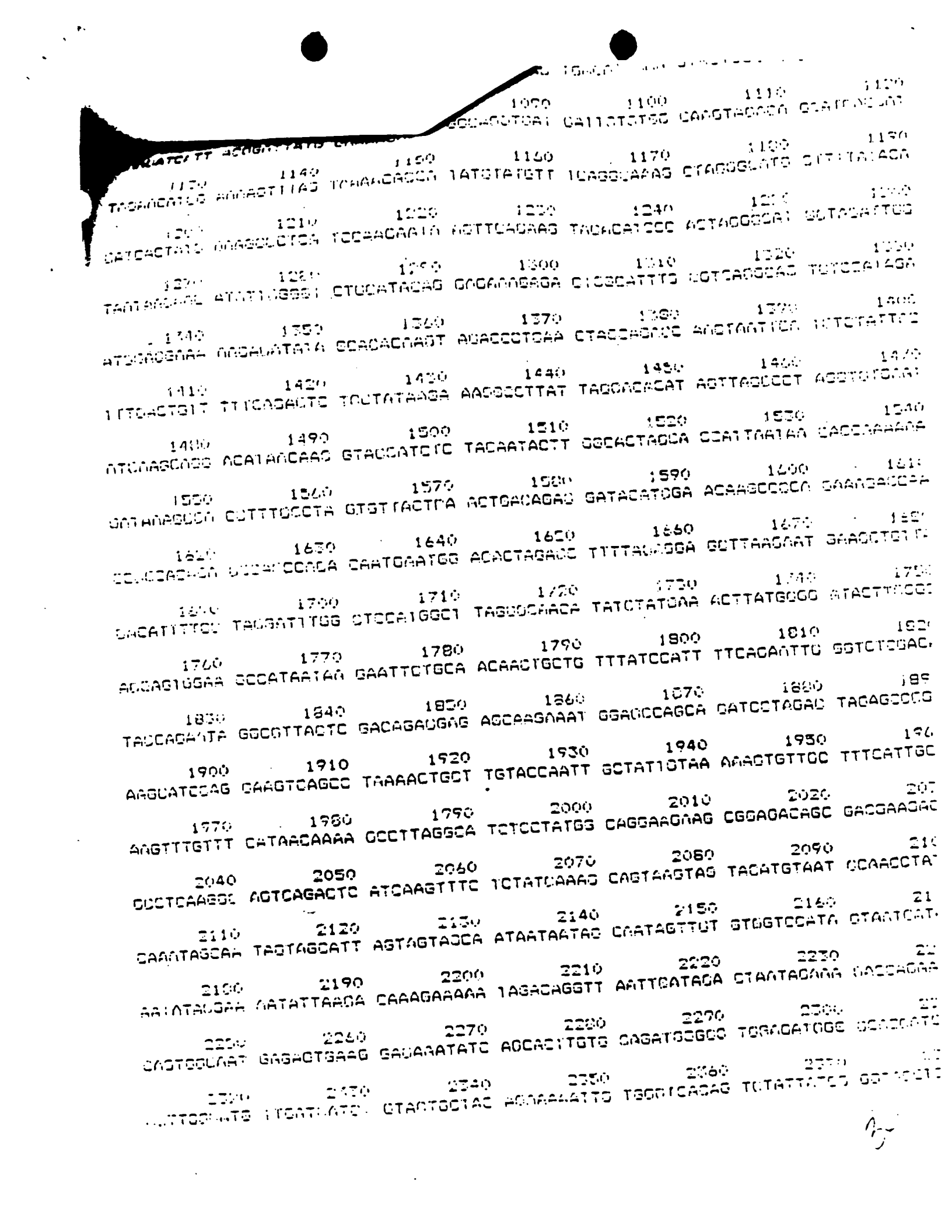

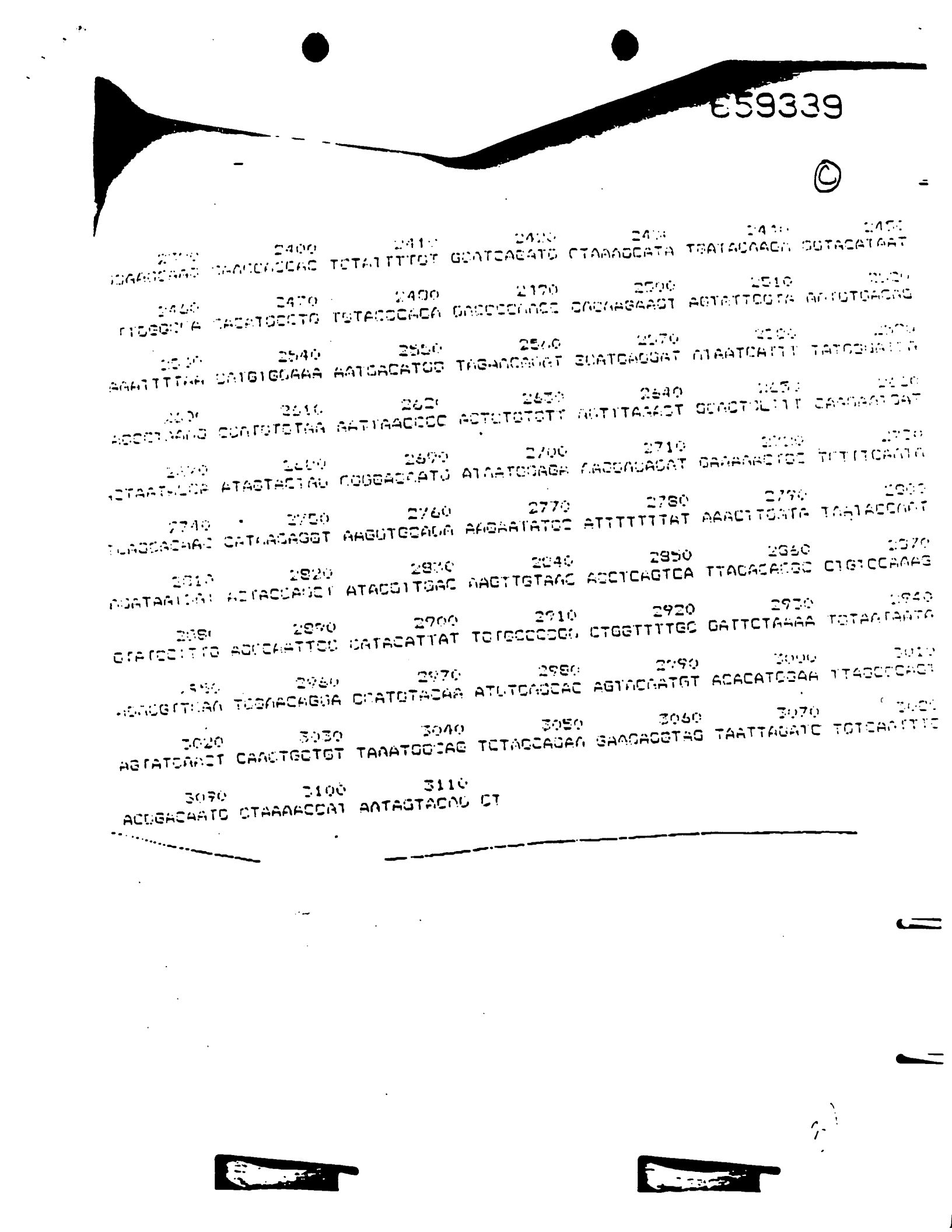

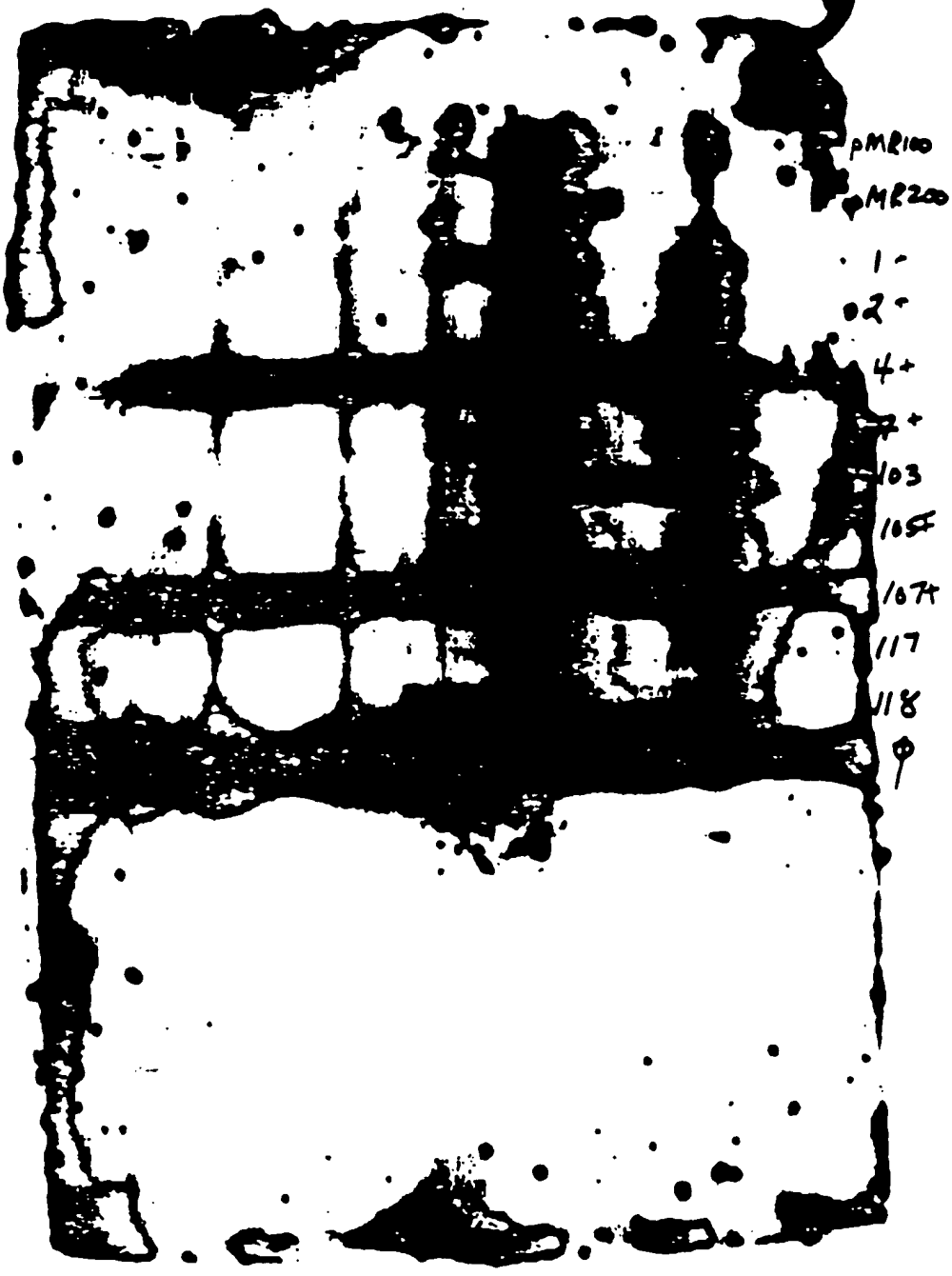

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